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Content

1. WELCOME TO THE WORLDLEISH7

Every four years, leishmaniacs from around the world gather in WorldLeish to discuss the latest advancements around these neglected tropical diseases and the seventh version was not an exception. In 2022, we had the participation of around 700 people, from 47 countries. Also, we had a great response from 536 students and professionals from around the world who sent us their abstracts to be part of the event as a poster or oral communications presentation and we are glad to say that we counted 195 oral presentations and 341 posters.

The experience and knowledge of the 210 speakers enriched the 44 Symposia, 8 Round Tables, 4 Special Meetings, 5 Plenary talks and 4 Successful stories that took place in those 6 days.

For Colombia and specifically the University of Antioquia, it was an honor to be the host of this Congress. And, for PECET, is a recognition for its almost 40 years of effort, research and hard work to treat leishmaniasis.

I would like to express my gratitude for your participation in this seventh version of the congress. Thanks to the knowledge and contributions, of all participants, it has been a complete success.

We know that it was not easy at all, however seeing all of you in Cartagena filled us with deep pride for the great challenge undertaken and the achievement reached.

May these events strengthen our "leishmaniac" spirit and recharge us to continue working in favor of this NTD.

Thank you very much.

With the expression of my admiration and respect.

Ivan Dario Vélez
Chair WorldLeish7

2. GENERAL SCHEDULE

MONDAY August 1st		Time	TUESDAY August 2nd	WEDNESDAY August 3rd	THURSDAY August 4th	FRIDAY 27 August 5th	Time	SATURDAY August 6th
		7:00 - 8:00	REGISTRATION	REGISTRATION	REGISTRATION	REGISTRATION		
		8:00 - 9:00	PLENARY TALK #1	PLENARY TALK #2	PLENARY TALK#3	PLENARY TALK #4	8:30 - 9:30	PLENARY TALK #5
		9:00 - 9:30	SUCCESSFUL STORY #1	SUCCESSFUL STORY #2	SUCCESSFUL STORY #3	SUCCESSFUL STORY #4	9.30 - 10:00	COFEE BREAK
		9:30 - 10:00	COFEE BREAK				10:00 - 11:30	SPECIAL MEETING #4
		10:00 - 11:30	SATELITE SYMPOSIUMS (sessions 1 - 5)	SATELITE SYMPOSIUMS (sessions 12-16)	SATELITE SYMPOSIUMS (sessions 23-27)	SATELITE SYMPOSIUMS (sessions 33 - 38)	11:30 - 12:00.	AWARDS
						SPECIAL MEETING #3		
		11:30 - 13:00	SATELITE SYMPOSIUMS (sessions 6 -11)	SATELITE SYMPOSIUMS (sessions 17 - 22)	SATELITE SYMPOSIUMS (sessions 28 - 44)	SATELITE SYMPOSIUMS (sessions 39 - 44)		
					SPECIAL MEETING #2			
		13:00 - 14:00	LUNCH	LUNCH	POSTER PRESENTATION Session 3	LUNCH	12:00 - 13:10	CLOSING LECTURE
14:00 - 19:00	REGISTRATION	14:00 - 15:30	SPECIAL MEETING #1	ROUND TABLE (1 - 4)	LUNCH/ FREE AFTERNOON	ROUND TABLE (5 - 8)	13:10 - 13:30	CLOSING REMARKS
		15:30 - 16:30	ORAL COMMUNICATIONS (sessions 1 - 7)	ORAL COMMUNICATIONS (sessions 15 - 21)		ORAL COMMUNICATIONS (sessions 29 - 35)		
		16: 30 - 17:30	POSTER PRESENTATION Session 1	POSTER PRESENTATION Session 2		POSTER PRESENTATION Session 4		
17:30 - 18.00	OPENING SESSION	17:30 - 18:00	COFEE BREAK			COFEE BREAK		
18:00 - 19:00	INAUGURAL LECTURE	18:00 - 19:00	ORAL COMMUNICATIONS (sessions 8 - 14)	ORAL COMMUNICATIONS (sessions 22 - 28)		ORAL COMMUNICATIONS (sessions 36 - 41)		
19:00 - 20:30	WELCOME RECEPTION							

3. SYMPOSIUMS

S1. ROLE OF ASYMPTOMATICS IN THE TRANSMISSION OF LEISHMANIASIS, SLEEPING SICKNESS AND CHAGAS DISEASE

S1-01: TOWARDS VL CONTROL. NEW DIAGNOSIS APPROACHES TO DETECT ASYMPTOMATIC PATIENTS

Eugenia Carrillo

WHO Collaborating Center for Leishmaniasis. National Center for Microbiology. Instituto de Salud Carlos III, Madrid, Spain. CIBER of infectious diseases. ecarrillo@isciii.es

The key control measures against leishmaniasis mainly rely on early case detection and chemotherapy. The identification and management of asymptomatic carriers has become a new and increasingly important challenge for visceral leishmaniasis control programs. Suitable and validated biomarkers are desirable in order to accurately identify the asymptomatic population in areas where *Leishmania* is endemic. They should be cost-effective, accurate, noninvasive and easily translatable to the field. To estimate the asymptomatic *Leishmania* infection, a combination of specific assays is recommended to avoid underestimation, such as a serological test (rK39, DAT), polymerase chain reaction (PCR)/ loop-mediated isothermal amplification (LAMP), and leishmanin skin test (LST). Recently, cytokine/chemokine quantification after the whole blood assay (WBA) has emerged to replace LST assessing cell-mediated immunity in field assays. *Leishmania*-specific IL-2 secretion after WBA has been proposed as a sensitive biomarker of asymptomatic *Leishmania infantum* infection in immunocompetent subjects while monokine induced-by-IFN-γ (MIG) was found to be a better marker of contact with *L. donovani*. Broadly, IP-10 was found to be an accurate global marker of asymptomatic subjects with positive cellular/humoral tests. These cellular biomarkers appear to

be reliable for detecting asymptomatic immune responders to *Leishmania* among HIV+ patients and patients receiving immunosuppressant drugs for autoimmune disease and solid organ transplant (SOT) recipients. Clearly, these immunossupressed patients in contact with *Leishmania* can mount a specific Th1 response against the parasite. Importantly, asymptomatic individuals that become candidates for a programmed immunosuppression for organ transplantation or therapy for autoimmune diseases could relapse and may require follow-up and surveillance for several years. As described, promising biomarkers and biomarker signatures are being discovered in leishmaniasis. Currently, it is needed to reach a consensus on how to define asymptomatic and on the tools to identify carriers in the field as the role they play in transmission may be of paramount importance for elimination/control programmes, mainly in immunosuppressed patients. Efficient validation and qualification process are requested before their evaluation in lengthy and costly trials.

Keywords *Leishmania*; VL; ASYMPTOMATIC; CYTOKINES; WBA; CONTROL

Financing CIBERINFECT; RICET (RD16CIII/0003/0002); AESI (PI18CIII/00029)

S1-02: CLINICAL AND EPIDEMIOLOGICAL IMPLICATIONS OF PEOPLE WITH *Trypanosoma cruzi* INFECTION WITHOUT ORGAN INVOLVEMENT

Maria-Jesus Pinazo[1], Sergio Sosa- Estani[2]

[1]Head of Chagas Program. DND*i*; [2]Regional Executive Director (LATAM). DND*i*

There are currently more than 40 million people globally at risk of acquiring *T. cruzi* infection, causative of Chagas disease, and more than six million people are estimated to be infected without diagnosis and treatment, people in the acute phase enter into a chronic phase. In the chronic stage of the disease, the most of them (70%) still remains without symptoms. This around five million people with *T. cruzi* infection will have parasites circulating in their blood and tissues for the rest of their lives without evidence of organ (cardiological/ digestive) involvement. Non-specific symptoms and low sensitivity tests to detect early organ damage are the only tools that clinicians have to classify *T. cruzi* infected people in symptomatic or asymptomatic. Even without evidence of organ damage, it is possible that a silent progressive physiopathogenic process is already in progress due to the presence of parasites circulating in the blood and those infected tissues where replication takes place. There are several ways by which circulating parasite could induce damage in tissues, even when it is not yet detectable (parasitological direct action, microvascular alterations and/ or specific immune responses, maintained in time due to infection chronicity). The first research need emerging from the discussion above is better diagnosis and classification of people with *T. cruzi* infection, and for this aim, more accurate tests to determine organ damage early should be available in countries with a high burden of Chagas disease. Biological markers of early organ damage, as well as prognostic biomarkers, are necessary to address this need. On the other hand, asymptomatic people may contribute to transmission in endemic and non-endemic countries primarily through mother to child transmission, transfusion of uncontrolled

blood and blood products, and organ transplant; perpetuating also the possibility of vector transmission in endemic countries. Early diagnosis and treatment of asymptomatic *T. cruzi* infection carries, is an individual and public health measure that contributes to disease control. The higher the burden of disease in a population in terms of parasitemia the greater the need for treatment. Nevertheless, there are structural barriers that difficult treatment scaling up, such as health workers' reluctance to prescribe treatment due to the safety profile, and the lack of clear evidence about the clinical benefit of treating asymptomatic adults. In Public Health terms, diagnosis and treatment of *T. cruzi*-infected asymptomatic people is a primary prevention for congenital and vectorial transmission and of secondary prevention in terms of morbimortality. Thus, to treat *T. cruzi* infection asymptomatic carriers could have a high impact giving the relevance of their role in the transmission and the benefit at an individual level to prevent irreversible organ damage, but is it important to consider the current knowledge on efficacy and toxicity of existing drugs. In the absence of other control tools such as vaccines, there is a need for safer drugs with good risk/benefit profiles in order to include treatment of non-symptomatic infected persons.

S2. NEW VACCINES AND IMMUNOTHERAPIES FOR CANINE LEISHMANIASIS

S2-01: LIVE ATTENUATED VACCINES FOR CANINE LEISHMANASIS

Thouraya Boussoffara[1], Kamaleshwar P Singh[2], Swarenendu Kaviraj[2], Ranadhir Dey[3], Sanjay Virakuti[4], Sreenivas Gannavaram[3], Wen-Wei Zhang[5], Patrick Lypaczewski[5], Shinjiro Hamanno[6], Greg Matlashewski[5], Sanjay Singh[2], Imen Labidi[7], Ifhem Chelbi[1,7], Elyes Zhioua[1,7], Abhay R. Satoskar[4], And Hira L. Nakhasi[3]

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Dogs are considered the main reservoir of *L. infantum* in zoonotic visceral leishmaniasis (ZVL) and the presence of infected dogs may increase the risk for human infection. Canine visceral leishmaniasis (CVL) is a major veterinary and public health problem in Southern Europe, North Africa and South America. The culling of seropositive dogs is not an effective control strategy against ZVL, and ethically it is not an acceptable method. Based on mathematical models, reducing the infectiousness of dogs to sand fly vectors is the most effective methods in controlling ZVL. Therefore, vaccination

against CVL remains the best alternative for controlling animal reservoirs. We have developed genetically modified *Leishmania* vaccines against *L. donovani* (causing VL) and *L. major* (causing cutaneous leishmaniasis) by deleting a centrin gene making them avirulent. We have tested the immunogenicity and efficacy of these two vaccine candidates either against needle infection in a controlled environment or against the natural sand fly mediated infection in the field. Vaccination with *LdCen*$^{-/-}$ induced immune response as revealed by the higher serological and lymphoproliferative T cell response. Further, *LdCen−/−* vaccinated dogs showed higher frequencies of activated CD4+ and CD8+ T cells, IFN-g production by CD8+ T cells, and increased secretion of TNF-α and IL-12/IL-23p40 and decreased secretion of IL-4. To explore the potential of *LdCen*$^{-/-}$ parasites as a vaccine candidate, we performed a 24-month follow up of *LdCen*$^{-/-}$ immunized dogs after needle challenge with virulent *Leishmania infantum*. Our data demonstrated that vaccination with a single dose of *LdCen*$^{-/-}$ (without any adjuvant) reduced up to 87.3% of parasite burden after 18 months of the virulent challenge. In addition, the protection was associated with induction of antibody production, T cell proliferative responses, as well as T cell activation. These studies demonstrated the potential of *LdCen*$^{-/-}$ vaccine to control *L. infantum* infection in dogs and may result in reduced transmission of CVL in the endemic areas. Recently, we have generated a dermotropic *Leishmania* vaccine using centrin gene deleted *L. major* (*L.major Cen*$^{-/-}$) to develop as a pan *Leishmania* vaccine. Our studies have demonstrated that immunization with *LmCen*$^{-/-}$ is safe and efficacious in appropriate animal models for CL and VL against both needle and sandfly mediated infection. We have manufactured *LmCen-/-* vaccine under Good Laboratory Practices (GLPs). Using GLP grade *LmCen*$^{-/-}$ vaccine we immunized dogs and exposed them in the field to infected sand flies harboring *L. infantum* in a CVL endemic focus. . Preliminary data shows that *LmCen*$^{-/-}$ parasites induce robust humoral and cellular responses and after two rounds of exposure to sand flies in the field, the immunized dogs showed enhanced immune response. These studies are ongoing, and the latest data will be discussed at the meeting. Taken together, our studies demonstrate that genetically modified parasites could be viable vaccine candidates for controlling dog infection and eventually controlling leishmaniasis in the endemic areas.

Keywords LIVE ATTENAUTED VACCINES, GLP GRADE, SAFE AND EFFICAICOUS, SANDFLY INFECTION, DOG VACCINE

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Communication Disclaimer: My contributions are informal communication and represents my own best judgment. These comments do not bind or obligate FDA

S2-02: AN EFFECTIVE NASAL NANOPARTICULATE IMMUNO-TREATMENT AGAINST LEISHMANIASIS IN DOGS

Rafael Antonio Do Nascimento Ramos[1], Alessio Giannelli[2], Angelo Scuotto[2], François Fasquelle[2], Didier Betbeder[2]

[1]Federal university of the agreste of Pernambuco, Brazil; [2]Vaxinano, Loos, France

Leishmaniasis are zoonosis caused by *Leishmania* sp. parasites and transmitted to human, dogs, and rodents by Phlebotomous sand fly. Depending on the parasite strain, the most common diseases' outcome are cutaneous incurable ulcers (CL) and/or visceral infection (VL). Leishmanium infantum infects both dogs and humans. The most used drugs in the treatment of leishmaniases in dogs are pentavalent antimonials such as sodium stibogluconate or Glucantime, paromomycin and miltefosine, or allopurinol. These treatments have many pitfalls such as high toxicity and side effects, decreasing efficacy due to resistance emergency and are pricey. To overcome these issues, here we propose a novel immuno-treatment based on maltodextrin-nanoparticles incorporating L. infantum antigens (NP-Linf). Thirty L. infantum positive dogs from an endemic region of North-eastern Brazil at stage 2 of infection (Leishvet.org) were recruited. We compared the efficiency of a Miltefosine treatment (10 dogs, 2 mg/kg, every day for 28 days) to nasal administrations of NP-Linf (10 dogs, 100µg at two weeks interval) and to the combination of the two treatments (10 dogs). The animals were evaluated for a period of 180 days, and they remained at home with their owners during the whole study period. The efficacy of each treatment against CL and VL was assessed all along the study by the microscopy of bone marrows and skins, as well as the IFAT evaluation (cut off = 1/40 dilution) at T0 and T180 days post treatment. NP-Linf intranasal administrations were well tolerated by dogs and no adverse effect occurred while in the Miltefosine group 4 dogs died during the treatment. Twelve weeks after the beginning of the study, no parasite was detected in the skin of dogs treated with NP-Linf. On the contrary, in the Miltefosine group 2 dogs

still had CL. IFAT analysis revealed that vaccinated dogs had a high decrease of antibodies in the serum. Altogether the results suggest higher efficacy of the nanoparticle-based immunotherapy in reducing the number of administrations (2 vs 28) without any side effect. These results suggest that further studies in order to develop this treatment can be envisaged to cure infected dogs. Further studies in term of following the cell mediated immunity induced by the immuno-treatment are planned.

Keywords LEISHMANIASIS; IMMUNOTHERAPEUTIC; DOGS; NASAL VACCINE

S3. EMERGING FOCI AND CHANGING EPIDEMIOLOGY OF LEISHMANIASIS

S3-01: OUR CHANGING VIEWS OF LEISHMANIASIS

Caryn Bern

University of California San Francisco, USA

The more we learn about the leishmaniases, the more this complex of diseases surprises us. Classic visceral species are found causing cutaneous leishmaniasis as well as the converse. The geography of leishmaniasis transmission changes over time, providing new challenges. The distinction between anthroponotic and zoonotic transmission cycles may be less fixed than previously assumed. And the most recently described group of species appears not to be transmitted by sand flies at all. In this symposium, we'll start with a brief overview of newly described aspects of leishmaniasis, followed by deep dives into three different aspects of emerging foci and surprising new epidemiological aspects of the leishmaniases.

S3-02: LEISHMANIASIS IN SRI LANKA: EMERGENCE AND EVOLUTION OVER 3 DECADES

Nadira D. Karunaweera, Sanath Senanayake, Samitha Ginige, Deepa Gamage, Guofa Zhou

Faculty of Medicine, University of Colombo, Sri Lanka

Leishmaniasis used to be reported as an exotic disease in Sri Lanka prior to 1990. Local transmission was considered non-existent then, although the disease was rampant in parts of neighbouring countries such as India, Bangladesh and Nepal. The first autochthonous case of cutaneous leishmaniasis (CL) was reported in Sri Lanka in 1992 with only sporadic cases until 2001, when the current outbreak started. There has been a steady increase in the numbers and spatial spread of leishmaniasis cases with cases recorded from all districts of the island. The majority of cases remains as cutaneious leishmaniasis and is due to a dermotropic variant of *L.donovani* with only a few cases of mucosal and visceral disease recorded. We analysed CL patient data since 2001, collected and maintained in databases at the diagnostic laboratory as well as at the central epidemiology unit, Ministry of Health that enabled the study of patient demographic, temporal and geographic trends and identification and monitoring of disease hotspots. A mixed spatio-temporal regression-autoregression model was used to study the influence of variables viz. environmental temperature, precipitation, neighbouring-district dispersal and infection carryover on disease dynamics and spatial distribution. Similar methods were also used to predict future distribution and trends of leishmaniasis cases. A total of 19,361 clinically confirmed leishmaniasis cases have been reported in Sri Lanka from 2001–2019. The disease affected all ages, from 1 to 81yrs, regardless of sex. However, there was a significant male preponderance (65%) and involvement of cases between 21 and 40 years (46%). In addition, we noted regional and temporal incidence differences for age and sex. Majority of lesions were on exposed areas of the body (90%), single and were small in size of <2 cm (65.0%). Both ulcerative and

nonulcerative stages of CL lesion were observed in nearly equal proportions (ulcerative: nonulcerative = 50.1: 49.9%); however, the proportions varied over time. Although fewer children (<14 years) appear to be affected, there were more males in that young age group that stood out. Majority of the lesions presented within the first 6 months of onset (74%), less than 10% presented after 12 months, and the pattern varied during the study period. Mean duration of a skin lesion was approximately 6 months. Based on the increasing pattern of case numbers, there were three phases: low-transmission phase (2001–2010), steady increasing phase (2011–2017), and outbreak phase (2018–2019). Based on case incidence dynamics the districts could be divided into three groups. The majority of districts were with low incidence (<2.5 cases per 1000 population), 4 districts with intermediate incidence (2.5 to 5 per 1000) and 7 districts with highest average annual incidence rates (>5 per 1000). We noted a progression in case rates, including a sharp rise in 2018, showing temporal expansion of disease-prevalent areas and 2 persistent hotspots. The northern hotspot, shifted and shrank over time unlike the southern hotspot that persistently expanded and remained spatially static. Risk analyses of the 7 districts with the highest incidence rates demonstrated that precipitation, neighbouring district effect and infection carryover effect were the factors that demonstrated the highest correlation with district-level incidence dynamics. The model predicted further intensification of disease transmission in future years with expansion of high transmission areas. Leishmaniasis case burden in Sri Lanka is steadily progressing and spatially expanding with distinct geographic patterns and disease hotspots. The situation calls for urgent attention of health authorities and policy makers to enable effective disease control through carefully planned interventions.

S3-03: CHANGES IN THE DEMOGRAPHY, EPIDEMIOLOGY AND CLINICAL PRESENTATION OF LEISHMANIASIS IN BRAZIL

Lucas P. Carvalho[1,2], Luís H. Guimarães[1,2], Albert Schriefer[2], Paulo R.L. Machado[1,2] and Edgar M. Carvalho[1,2]

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Leishmania braziliensis is the most prevalent species causing tegumentary leishmaniasis in Brazil. Corte de Pedra, a district in Bahia state, Northeastern Brazil, is an area of *L. braziliensis* transmission, with average of 1,000 cases of tegumentary leishmaniasis diagnosed per year. Cutaneous leishmaniasis (CL) is the most prevalent clinical form of the disease, occurring in 90% of the cases. Mucosal (ML) is diagnosed in 3% of the cases concomitantly or after CL. Disseminated leishmaniasis (DL), characterized by more than 10 up to more than 1,000 papular, acneiform and ulcerated lesions, is an emerging form of the infection with a 30-fold increase of prevalence in the last 25 years. About 1% of the patients present atypical lesions as verrucous, multiple nodular lesions in one area of the body, or large ulcers with non-limited borders. Moreover, about 20% of subjects living in this area, and without previous history of CL, have a subclinical infection. It is not clear what is the wild reservoir of *L. braziliensis,* but about 20% of the dogs have evidence of *L. braziliensis* infection detected by PCR in skin biopsies, and the ratio for infections/disease in dogs is 7:1. Canine tegumentary leishmaniasis is characterized by un ulcer similar to the one observed in humans with CL. *L. braziliensis* and *L. amazonensis* where the two species causing tegumentary leishmaniasis in the past, however for more than 20 years *L. braziliensis* has been the only species detected in this area. *L. braziliensis* is polymorphic in this region and genotypic differences among parasites of the same species is associated with the different clinical forms, severity of the disease and high rate of failure to therapy. Over a 20 years-period, there was an increase in tegumentary leishmaniasis in children and in individuals with more than 60 years of age, suggesting peridomiciliary and domiciliary

transmission. CL, ML and DL patients share the same immunopathogenesis features, characterized by an exaggerated inflammatory response and low number of parasites. High levels of proinflammatory cytokines, TNF, IFN-, IL-17, IL-1 and IL-6 participate of the pathology, but cytotoxicity mediated by NK and CD8+ T cells have been considered the most important immunological responses associated with tissue damage in *L. braziliensis* infection. Although significant differences in immune response may not be linked to the occurrence of the different clinical forms, high levels of IL-1 and high parasite burden is associated with longer time to heal and therapeutic failure to pentavalent antimony.

S3-04: LEISHMANIASIS WITHOUT SAND FLIES: THE UNFOLDING STORY OF *Mundinia*

Paul A Bates

Division of Biomedical and Life Sciences, Lancaster University, United Kindom

Ongoing research on genus *Leishmania* has recently seen the emergence of a new subgenus of parasites, the *L. (Mundinia)*, alongside the other three established subgenera, *L. (Leishmania)*, *L. (Viannia)* and *L. (Sauroleishmania).* The majority of reported human infections arise from parasites in the subgenera *L. (Leishmania)* and *L. (Viannia)*. However, the new subgenus *Mundinia* does contain human pathogens, these being *L. (M.) martiniquensis*, which is widely distributed, *L. (M.) orientalis* from Thailand and the recently described species *L. (M.) chancei* from Ghana. Species non-pathogenic to humans in subgenus *Mundinia* include the well-known *L. (M.) enriettii* from Brazil, *L. (M.) macropodum* from Australia and the recently described *L. (M.) procaviensis* from Namibia. There are several aspects of the biology of the subgenus *Mundinia* that make them particularly fascinating, and arguably also important for the proper understanding of the transmission and pathology of human leishmaniasis in general. The first is that phylogenetic analysis indicates that the *Mundinia* sit at the base of the *Leishmania* clade and are the earliest branching subgenus. So, we may consider them to be modern day representatives of ancestral *Leishmania*. This may partially explain their wide geographical distribution and interesting variation in mammalian hosts. Second, as the subgenus contains both human pathogens and non-pathogenic species, comparative studies can aim to understand the basis of disease and pathology in human and other hosts. Third, and perhaps of most interest, are the potentially unusual vectors of *Mundinia*. The first evidence for this came from observations on *L. macropodum*, in which *Forcipomyia* midges were found to support mature infections of the parasite. Subsequent laboratory investigations have shown that *Culicoides* midges are better experimental hosts than the various

phlebotomine sand flies tested to date. Recently there have been additional observations of *Leishmania*-infected midges from field studies. Currently, the role of midges as vectors for some or all *L. (Mundinia)* species is not proven beyond doubt. There is still the possibility of undescribed sand fly species responsible for transmission. However, the weight of current evidence certainly supports the "midge hypothesis". Either way, the vectors of *L. (Mundinia)* will make an important contribution to understanding the transmission of leishmaniasis and may even require a redefinition of genus *Leishmania* itself.

S4. ELIMINATING VL AS A PUBLIC HEALTH PROBLEM IN THE WHO SOUTH-EAST ASIA REGION: THE LAST MILE CHALLENGES AND OPPORTUNITIES THROUGH THE NEW REGIONAL STRATEGY

S4-01: PROGRESS, ACHIEVEMENTS, REMAINING CHALLENGES AND THE NEW REGIONAL STRATEGY FOR VL ELIMINATION IN THE WHO SOUTH-EAST ASIA REGION

Aya Yajima

Regional Adviser – NTD WHO SEARO, New Delhi, India

Visceral leishmaniasis (VL or also called kala-azar) is endemic in three countries (Bangladesh, India and Nepal) of the WHO South-East Asia Region with an estimated 147 million people at risk of infection. Bhutan and Thailand also report sporadic cases of VL. In 2005, the Regional kala-azar elimination initiative was launched where the governments of Bangladesh, India and Nepal signed a memorandum of understanding to cooperate and jointly achieve kala-azar elimination by 2015, defined as an annual incidence of less than 1 case of VL per 10,000 population. The Regional Strategic Framework for Elimination of Kala-azar from the South-East Asia Region 2011-2015 was launched in 2012. The elimination initiative was further joined by Thailand and Bhutan in 2014. As a result, the South-East Asia region has made a significant progress in elimination of kala-azar. The number of reported kala-azar cases in the three endemic countries has declined steadily from over 50,000 cases in 2007 to 2,335 cases in 2020. By the end of 2021, cases have further decreased to 1577 (provisional data) and 746 (99%) of 756 implementation units (IU) in the Region (i.e. sub-district in Bangladesh and India and district in Nepal) having achieved the elimination target and only 10 IUs (1%) remain above the elimination threshold. Specifically, the elimination target for kala-azar was reportedly

achieved in all endemic upazilas of Bangladesh, 99 % of blocks in India and 87 % of endemic districts in Nepal. Bangladesh has sustained the target of less than one VL case per 10, 000 population in all the IUs since 2017. In 2020, WHO launched the new global NTD Roadmap "Ending the neglect to attain the Sustainable Development Goals: a road map for neglected tropical diseases 2021–2030". This roadmap clearly states the elimination of VL as a public health problem in the South-East Asia Region as one of its global targets, based on the fact that such achievement is considered feasible only in the South-East Asia Region at present. Given the substantial progress made in the last decade since the launch of the previous Regional Strategic Framework for Elimination of Kala-azar from the South-East Asia Region in 2012 and reiteration of the Regional elimination as one of the global targets for VL elimination, WHO South-East Asia Regional Office (SEARO) is launching a new Regional Strategy for elimination of kala-azar to reinforce the Regional initiative with its focus on integration and sustainability to accelerate and sustain elimination of kala-azar in the South-East Asia Region.

S4-02: ACHIEVEMENTS, CHALLENGES AND REASSESSING VL ENDEMICITY IN NON-PROGRAM DISTRICTS IN NEPAL

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Visceral leishmaniasis (VL), also called kala-azar, was first officially recorded in Nepal in 1980 from one district. By now it has become endemic in 23 districts, with an estimated fifteen million population at risk of infection. VL is caused by *Leishmania donovani* and is transmitted by *Phlebotomus argentipes,* with humans being the only known reservoir. In 2005, the Regional Kala-azar Elimination Initiative was launched in which the government of Nepal along with Bangladesh and India signed a memorandum of understanding to cooperate and jointly achieve VL elimination as a public health problem by 2015, a target defined as an annual incidence of less than 1 case of VL per 10,000 population. Since the start of the Initiative, Nepal has made a significant progress towards the elimination of VL. The number of reported VL cases has declined steadily from 1564 cases in 2005 to 252 in 2021. Nepal was the first country to reach the target threshold for elimination in all endemic districts in 2013, and has sustained this target in all endemic districts ever since. Despite this remarkable progress, elimination of VL has not yet been certified in Nepal, as several challenges continue to prevent a sustainable elimination in the long run. The threshold of elimination was surpassed in one supposedly non-endemic district in 2019 and a several more (supposedly non-endemic) districts since then. A geographical expansion of VL cases has also been observed in recent years, with more than half of the total VL cases now being reported from districts hitherto considered to be non-endemic. In addition, an increasing number of relapse is being observed in pediatric VL cases in the west of the country, together with increasing numbers of (muco-) cutaneous

leishmaniasis cases, many of whom without a travel history, indicating local transmission of either CL-causing *L. donovani* or even another species of *Leishmania* responsible for (M)CL. Although Nepal is close to VL elimination, sustaining and consolidating the gains made since the start of the Elimination Initiative will be another challenge.

S4-03: CURRENT PROGRESS AND SUCCESS OF THE NATIONAL KALA-AZAR ELIMINATION PROGRAM OF BANGLADESH

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Bangladesh signed a memorandum of understanding (MoU) in 2005 in collaboration with India and Nepal to eliminate the Kala-azar from their respective countries by 2015, which was later extended to 2017. Where the elimination target was set to reduce cases to less than one per 10,000 population at Upazila (sub-district) level. Bangladesh reached the above target in 2016. Now, Bangladesh is working on the formal declaration of Public Health Elimination of the disease, for which dossier submission is essential to the World Health Organization (WHO). The national Kala-azar elimination program (NKEP) is moving forward to achieve zero transmission by 2025 and achieve Kala-azar-free status by 2030. Since 2008, the NKEP operating all core functions that are aligned with regional strategy. The country has initiated its core interventions following the five strategies which were defined by the WHO to combat the disease. Core interventions include massive training for health care professionals (doctor, nurse, medical technologist, statistician, health inspector, assistant health inspector and health assistant), prompt diagnosis and effective treatment including 12 months post-treatment follow-up, integrated vector management through indoor residual spraying and donation of a long-lasting insecticide-treated bed net, disease surveillance through DHIS2, active case detection and community sensitization. At the beginning of the Elimination initiative, cases were diagnosed by aldehyde (AT) and direct

agglutination test (DAT), and sodium stibogluconate was used for treating the patients. The rapid diagnostic test, rK39 was introduced for diagnosis of cases in 2009 and the oral drug, Miltefosine. Vector control activities were started in 2012 by indoor residual spraying using deltamethrin 5WP. At the same time, community sensitization activities were undertaken to create public awareness for seeking proper health care and support vector control activities. Single-dose liposomal amphotericin B (AmBisome) was introduced in 2014 as the first line of treatment for new kala-azar cases and Miltefosine was used for treating post kala-azar dermal leishmaniasis (PKDL) cases. Active case detection by different methods was also introduced at the same time. Live data collection of treated cases through DHIS2 was started in 2015; earlier, it was either over phone or e-mail or by post office sending the hard copy at central level. Bangladesh has 100 program (endemic) Upazilas where all interventions were provided; however, sporadic cases from non-program Upazilas have received diagnosis and treatment facilities free of cost. Finally, the country reached the elimination target in 2016 in all Upazilas of below one case per 10,000 population and the total reported cases was 454. This achievement was verified by a joint (national and international expert) monitoring mission. Between 2017 and 2021, 380, 290, 224, 132, and 99 cases were reported by the country respectively. At the beginning, the national elimination program faced many challenges in operationalizing the program activities, later gradually overcame those challenges and reached the target of eliminating one case per 10,000 population at the Upazila level in all 100 program Upazilas. However, to sustain the achievement, continuous effort is needed and the country will be reached its goal of kala-azar free Bangladesh by 2030.

S4-04: ELIMINATING VL AS A PUBLIC HEALTH PROBLEM IN THE WHO SOUTH-EAST ASIA REGION: THE LAST MILE CHALLENGES AND OPPORTUNITIES THROUGH THE NEW REGIONAL STRATEGY

Be-Nazir Ahmed

Country Lead ASCEND Bangladesh, Former Line Director, National Kala-azar Elimination Program, CDC, DGHS, MOHFW

Kala-azar, the vector borne parasitic disease has been endemic in south Asian countries for centuries claiming hundreds of lives annually. Transmission in the sub-continent generally occurs in rural areas with a heavy annual rainfall, with a mean humidity above 70%, a temperature range of 15-38 °C, abundant vegetation, subsoil water and alluvial soil. The disease is most common in agricultural villages where houses are frequently constructed with mud walls and earthen floors, and cattle and other livestock live close to humans. Earliest recorded outbreak of fever ascribed to Kala-azar was reported in 1824-25 in Jessore (Now in Bangladesh) which caused deaths of no less than 7,50,000 people during a period of three years. Prevalence of kala-azar was recognized in the beginning of 20th century in south India also. Subsequently the disease was reported in Nadia (WB) in 1832-33 & in Hooghly in 1857. Kala-azar outbreaks in erstwhile Calcutta were reported after famine of 1943 & reached its peak in 1946. But concerted efforts by three countries, India, Bangladesh and Nepal through a MoU have changed the scenario reducing the global burden shared by SEAR from 70% in 2005 to 18% in 2020. Bangladesh has achieved elimination in all 100 endemic districts in 2017 and has been maintaining it, India with only 7 and Nepal only 3 IUs to achieve that. With this background the region has the last mile of challenges to achieve elimination and sustain with reporting of VL case beyond IUs specially in Nepal, the real threat of re-emergence, growing number of CL specially in Sri Lanka, challenge for adequate funding, detection of remaining cases and increase in vector density following cessation of vector control measures. The region has opportunities also through a bunch of VL experts and continuation of

political commitments for NTDs including VL following the Global NTD roadmap. The region has started the effort to draft a new VL strategy to consolidate the achievement and face the emerging challenges. The goal of the proposed regional strategy is to achieve and sustain elimination of VL as a public health problem in the SEAR. The objectives are to ensure early case detection and complete case management and to reduce density of sandfly vectors. The cross cutting areas supporting achievement of strategic objectives are strengthening governance and programme management, strengthening governance and programme management, strengthening and sustaining health workforce and laboratory capacity and referral system, improving inventory management system and storage conditions, enhancing advocacy, regional partnership and cross-border collaboration and continue to catalyze innovation and research.

S5-05: ACHIEVEMENTS, CHALLENGES AND REASSESSING VL ENDEMICITY IN ENDEMIC DOUBTFUL DISTRICTS IN NEPAL

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Visceral leishmaniasis (VL), also called kala-azar, was first officially recorded in Nepal in 1980 from one district. By now it has become endemic in 23 districts, with an estimated fifteen million population at risk of infection. VL is caused by *Leishmania donovani* and is transmitted by *Phlebotomus argentipes,* with humans being the only known reservoir. In 2005, the Regional Kala-azar Elimination Initiative was launched in which the government of Nepal along with Bangladesh and India signed a memorandum of understanding to cooperate and jointly achieve VL elimination as a public health problem by 2015, a target defined as an annual incidence of less than 1 case of VL per 10,000 population. Since the start of the Initiative, Nepal has made a significant progress towards the elimination of VL but the pace of elimination is stalled with in the last half decade. Ample numbers of cases are reported from remote community of western hilly districts in recent year (101 of 257 cases in 2021) and are reported up to from 2960-meter elevation. Nepal was the first country to reach the target threshold for elimination in all endemic districts in 2013, and has sustained this target at national level. However, the elimination threshold has been crossed by endemic doubtful as well as endemic districts since 2016. Despite this remarkable progress, elimination of VL has not yet been certified in Nepal, as several challenges continue to prevent a sustainable elimination in the long run. The threshold of elimination was surpassed in one supposedly non-endemic district (Dolpa) in 2019 and followed by endemic districts in 2020 and 2021 (Okhaldhunga, Kalikot and Bajura in 2021). A geographical expansion of VL cases has also been observed in recent years, with more than one third of the total VL cases now being reported from these districts

hitherto considered to be endemic doubtful. In last three years, an increment of pediatric VL cases has been observed (57/204, 72/215 and 98/257 cases in 2019, 2020 and 2021 respectively) with nearly two third of pediatric VL cases being reported from western parts of country. In addition, an increasing number of relapse is being observed in pediatric VL cases in the west of the country, together with increasing numbers of (muco-) cutaneous leishmaniasis cases, many of whom without a travel history, indicating local transmission of either CL-causing *L. donovani* or even another species of *Leishmania* responsible for (M)CL. Although Nepal is close to VL elimination, sustaining and consolidating the gains made since the start of the Elimination Initiative will be another challenge.

S5. INFLAMASOMES AND *Leishmania*

S5-01: INNATE IMMUNE SENSORS AND INFLAMMASOMES IN THE *Leishmania*-MACROPHAGE INTERACTION

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Inflammasomes are multimeric protein complexes that assemble in the cytosol of many types of cells, including innate immune cells. The inflammasomes can be activated in response to infection or in response to stress signals that induce damage in the host cell membranes. These platforms trigger inflammatory processes, cell death, and the control of microbial replication. Many inflammasomes have been described so far, including NLRP3, NAIP/NLRC4, caspase-11, and AIM2. The ligand for NLRP3 is still unidentified, but the efflux of K+ is essential for NLRP3 activation. By contrast, inflammasomes, such as those composed of NAIP/NLRC4, caspase-11, and AIM2, can be activated by bacterial flagellin, LPS, and dsDNA. The knowledge of inflammasome biology in response to bacteria has advanced tremendously in the last decade, while inflammasome activation in response to intercellular parasites, remains less explored. *Leishmania* infection trigger activation of the NLRP3 inflammasome in macrophages for restriction of intracellular parasite replication. Accordingly, *Leishmania* can dampen NLRP3 activation as an evasion strategy. In vivo, the NLRP3 inflammasome can promote parasite clearance, but the failure to eliminate parasites in the tissues together with sustained inflammasome activation can promote IL-1β-mediated disease pathology. Data to be presented will highlight recent data regarding activation and consequences of

inflammasome activation in response to *Leishmania*, a process that effectively impacts the development of the disease in humans.

S5-02: CD8 T CELLS PROMOTE NLRP3 INFLAMMASOME ACTIVATION AND IL-1β RELEASE IN CUTANEOUS LEISHMANIASIS

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The host immune response plays a critical role not only in protection from human leishmaniasis, but also in promoting disease severity. We have described a pathological role for $CD8^+$ T cells in the skin lesions caused by *Leishmania braziliensis*. Using experimental models of infection and skin samples from infected patients, we found that cytotoxicity induced by $CD8^+$ T cells is a major mediator of immunopathology in cutaneous leishmaniasis. However, the downstream mechanisms of cytolytic activity that cause disease severity were unclear. We hypothesized that dying cells targeted by cytolytic $CD8^+$ T cells release damage associated molecular patterns that activate the inflammasome with the consequent release of the pro-inflammatory cytokine IL-1β. Using experimental models of cutaneous leishmaniasis, we observed that cytotoxic $CD8^+$ T cells induce inflammasome activation as measured by IL-1 protein production and active caspase-1 expression in the skin of infected mice. The increase in IL-1 expression and immunopathology were dependent on commensal bacteria present in the skin, since GF mice did not develop severe disease and had significantly less IL-1 expression in the skin in comparison to SPF mice. We also found increased production of IL-1 by cells from the lesions of *L. braziliensis* patients, and IL-1β levels correlated with granzyme B produced by $CD8^+$ T cells. IL-1β blockade, but not IL-1a blockade, prevented the pathology induced by $CD8^+$ T cells in vivo. In addition, Caspase 1/11 and NLRP3 deficient mice did not develop severe pathology induced by $CD8^+$ T cells. Inhibiting IL-1β signaling or the NLRP3 inflammasome with pharmacological inhibitors prevented exacerbated pathology in mice. Importantly, IL-1b acted in a feed-forward loop and was necessary to induce

the development of pathogenic $CD8^+$ T cells. In summary, our findings demonstrate that inflammasome activation and IL-1β release are a consequence of cytotoxicity induced by $CD8^+$ T cells and cause severe disease.

S5-03: IMMUNE CIRCUITRIES THAT MAINTAIN DERMIS RESIDENT MACROPHAGES AS REPLICATIVE NICHES FOR *Leishmania major* IN A STRONG Th1 ENVIRONMENT

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Recent advances have revealed that many tissue resident macrophages are embryonically seeded, self-renewed, and perform homeostatic functions associated with M2-like activation programs. We provide evidence that a mannose receptor high, MHCII negative (MHC^{-}MR^{hi}) population of dermis resident macrophages (DRM) maintain their M2-like phenotype even in the strong Th1 immune environment produced in response to *Leishmania major* infection in the skin of conventionally resistant C57Bl/6 mice. The DRM are the predominant infected cell type at 1 hr and 24 hr following *L. major* transmission by sand fly bite, and in some cases can be visualized by intravital microscopy acquiring their infection via transfer from or efferocytosis of parasitized neutrophils, providing direct evidence for the "Trojan Horse" model. Both DRM and monocyte-derived cells are infected by all *L. major* strains tested, but the preferential infection of DRM by some strains throughout the course of infection is responsible for their non-healing phenotype. The DRM are not replaced by blood precursors during infection, but are locally maintained by IL-4 and IL-10. Eosinophils are a major source of localized IL-4 production in cutaneous lesions, and in mice conditionally deficient in IL4/IL13 from eosinophils, the number of DRM is reduced, their activation program shifts to a proinflammatory state, and the course of cutaneous disease is attenuated. IL-4–stimulated DRM, in concert with IL-10, produce a large amount of CCL24 (eotaxin), which functions to promote eosinophil influx and their interaction with DRM. By single cell RNA seq analysis, DRM are also the sole source of the alarmin thymic stromal lymphopoietin (TSLP) to activate innate lymphoid cells 2 (ILC2), which produce IL-5 to amplify eosinophil-DRM cooperative interactions. IL-

1 beta also contributes to ILC2 function, and mice deficient in IL-1R signaling and caspase-1 activation maintain fewer DRM during infection and display an attenuated course of disease. Thus, in the setting of the strong pro-inflammatory environment of the *L. major* loaded dermis, any disruption of the localized type 2 response circuitries that maintain the M2-like phenotype of DRM, will alter their number and activation states, and promote stronger resistance to *L. major* infection.

S6. PATHOGENESIS OF KALA-AZAR

S6-01: PATHOGENESIS OF VISCERAL LEISHMANIASIS: THE DOG MODEL

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Initially, VL was considered an eminently rural disease, but more recently, it has expanded to medium and large urban areas and has become a growing public health problem in the country and in other areas of the American continent. Due to its proximity to humans, the dog has been pointed as the main reservoir of *L. infantum* in China, the Mediterranean basin and the Americas. In addition to the importance of the dog as a reservoir, canine disease is also considered as valuable for the understanding of human disease. The clinical picture presented in canine disease is similar to the picture observed in human disease, with except for the cutaneous involvement observed in canine disease, which is more exacerbated than that observed in human disease. In addition, the natural history of canine disease has many similarities with human disease, which makes canine VL be an important model of study for human LV. An important aspect to be observed is the diversity of possible presentations of canine disease. Infected dogs may present from no clinical signs, and these animals are called asymptomatic, until a high number of signs, considered polysymptomatic. Due to this polymorphism, it has been sought to identify possible disease markers for the disease in order to define the profile of resistant animals and animals susceptible to visceral leishmaniasis. To date, some parameters have been used as markers of susceptibility and resistance, such as the presence of parasites in lymph nodes and other organs, such as the spleen, determined through culture. Identification of the parasite in tissue culture of the host indicates an inability of the host to control the proliferation of *Leishmania* in the organism, and consequently,

to control the infection. The presence of clinical signs is an important marker. Animals with resistance to infection tend to remain asymptomatic for long periods, and may never show signs of disease, whereas susceptible animals present a series of clinical signs. However, the great variety and lack of specificity of the clinical signs attributed to VL makes it difficult to evaluate. Another important marker of resistance or susceptibility to visceral leishmaniasis is the result in the leishmanin skin test (LST). The positive LST result indicates that the animal produces a cellular response when in contact with the *Leishmania* antigen, and since immunity to the parasite is mediated by T cells, a positive cellular response may indicate a resistance profile to infection. An aspect to be evaluated is the production of specific antibodies. Asymptomatic animals have been shown to produce IgG1 in greater amounts, while symptomatic animals produce more IgA, IgE and IgG2. It was also observed that animals with higher levels of IgG1 present lower parasite burden in the bone marrow, whereas animals with higher levels of IgA, IgM and IgG2 present higher parasitic burden in organs such as skin, bone marrow, lymph node and spleen. Our group demonstrated that there is an association between the presence of severity markers and the disruption of the spleen lymphoid tissue observed in VL. Animals with splenic parasitism identified in culture and negative result in LST showed more often the disruption of the splenic lymphoid tissue than those animals that did not present splenic parasitism and had a positive result in LST. These findings, together with the fact that the spleen is a highly parasitic organ in animals with susceptibility profile to VL, show that the spleen is an organ of great importance in the context of LV. Thanks to the large number of naturally infected dogs in regions where LV is endemic, there is the possibility of different analyzes of the markers mentioned above. Such analyzes and their correlations with data from human studies make the dog an excellent study model for VL, and the understanding of the pathogenesis of canine disease becomes of great value for the understanding of the disease in humans, also contributing for advances in the search for better alternatives in the treatment of the disease.

Keywords DOG; VISCERAL LEISHMANIASIS; PATHOGENESIS; DISEASE MODEL.

S6-02: THE PATHOGENESIS OF KALA AZAR - THE MOUSE MODEL

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Over several decades, mice have played a pivotal role in the study of leishmaniasis, and lessons learnt from the study of experimental *Leishmania* infections in mice have significantly enriched our fundamental understanding of host immune mechanisms at the molecular, cellular and tissue level. Nevertheless, as translational efforts increase, and the concept of "disease positioning" becomes more prevailing, it is appropriate to question how well mice reflect clinical kala azar. In this presentation, I will discuss what we have learnt from the use of mouse models of *L. donovani* and *L. infantum* infections, what we may learn in the future through the application of new mouse genetic models and importantly, where clinical research may have been mis-directed by an over-interpretation of the results generated in mouse models.

S6-03: HUMAN VISCERAL LEISHMANIASIS: FROM EXPOSURE TO DISEASE

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The disease is an exception after exposure to viscerotropic *Leishmania*. Indeed, a variable proportion of humans in an area where visceral leishmaniasis (VL) is endemic test positive for past *Leishmania* infection, but just a few have a history of the disease. One study calculated more than 200:1 the proportion of asymptomatic to symptomatic infection. After a sandfly bite, most inoculated *Leishmania* metacyclic promastigotes die in a few minutes by human complement lysis before entering into neutrophils or monocytes. Some parasites that successfully penetrate the epidermis alive meet neutrophils and mononuclear cells. They may accomplish their fate as mononuclear intracellular parasites and establish the infection if they are internalized. Infected neutrophils may present antigens directly to T-cells or start apoptosis and are phagocytized. Then, the infected cells migrate to secondary lymphoid organs and **visceralize** the infection. After visceralization, containment may occur but seems infrequent, as seen by the rarity of self-cure in symptomatic or oligosymptomatic patients. Several studies have demonstrated that the host's genetic background may play a role in the fate of the established infection. If the immunity generated by the previous disease or past asymptomatic infection is strong, reactivation or reinfection is rare in immunocompetent humans. However, acquired immunosuppression strongly influences the evolution of infection from asymptomatic to symptomatic. Additional relevant information regarding the bias of disease incidence towards the male sex, but not of infection incidence, is modulated by dihydrotestosterone. Not only host factors, like acquired immunosuppression, determine the success of infection. Likewise, parasite factors may also contribute to visceralization. Indeed, the A2 gene family and a mutation in the ras-like RagC GTPase enzyme in the mTOR pathway seem to contribute to the attenuation as proposed for the

visceralization of cutaneous leishmaniasis caused by *L. donovani* in Sri Lanka. Finally, whole-genome sequencing of *L. infantum* has revealed that the parasite genome contributes to the mortality of patients with VL. Besides the parasite and the vertebrate host, sandflies can also modulate the infection's success when delivering infective promastigotes into the skin together with saliva. Sandfly saliva is rich in pharmacologic active substances acting on vasodilation, coagulation, and immune response to *Leishmania*. After successful infection and disease installation, INF-g, other pro-inflammatory cytokines, and IL-10 are elevated in the blood. However, the specific acquired response does not develop, as seen by the failure of T-cells to proliferate and secrete INF-g upon exposure to parasite antigens. The conclusion is straight: parasite replication becomes out of control and is followed by innate systemic response via pattern recognition receptors (PRR). Since *Leishmania* has no toxin to harm any parenchymal host cells or tissues, the innate host response fully mediates the disease, except hepatosplenomegaly. After this point, signs and symptoms develop.

- Hepatosplenomegaly is almost universally found in patients with VL. It is determined by lymphoid hyperplasia, as histopathological studies have revealed that both organs are full of parasites. There is no congestion to explain splenomegaly. Albeit marked atrophy of the splenic white pulp associated with necrosis and fibrosis of thymus-dependent areas, there is an accumulation of parasites-containing macrophages and plasma-cell hyperplasia, determining the increased spleen volume. However, some degree of hypertrophy is seen in the liver by the proliferation of Kupffer cells. VL's signs and symptoms overlap with the effects of IL-6 but also have similarities with those of IL-1b, TNF-a, IL-8, and other pro-inflammatory cytokines. IL-6 has the broadest effect due to its ligands. After *Leishmania* molecules interact with PRR triggering signal transduction on macrophages, pro-inflammatory and anti-inflammatory cytokines are secreted. The diversity of IL-6 actions relates directly to interaction with both membrane-bound and soluble IL-6 receptors, and the co-receptor gp130. In hepatocytes, IL-6 link with membrane-bound and gp130 leads to the synthesis of the acute phase reactants (APR) and, in monocytes, leads to overexpression of tissue factor, triggering the coagulation cascade. The gp130 co-receptor is expressed on the plasma membrane of most cell types,

including the hypothalamus and muscle cells. APR is, therefore, a cornerstone to the development of most symptoms of VL. The positive APR includes erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), hepcidin, procalcitonin, CD14, serum amyloid A protein, fibrinogen, ferritin, and complement proteins C3 and C4, among others, are increased in VL. The level of response varies among different APRs. CRP is highly correlated with IL-6, can increase a few thousand folds in response, and is very high in VL. Albumin, typically low in VL, decreases during the inflammation response and is called negative APR with others. ESR, almost always high in VL, is a nonprotein APR that changes in response to plasma fibrinogen levels and plasma viscosity and hence is an "indirect" APR. Hepatocytes also release pro-coagulant proteins, fueling coagulation.

- Fever is the most frequent symptom of VL. It is mediated by inflammatory cytokines (endogenous pyrogens, mostly IL-1b and IL-6), triggering the release of prostaglandin A2 (PGE2) acting in the preoptic area of the hypothalamus via the prostaglandin E receptor 3 (EP3). Endogenous pyrogens are then synthesized and released after PRR (likely Toll-like receptor 2) interaction with *Leishmania* in macrophages, leading to fever.

- Weight loss is also a ubiquitous symptom of VL. Persistent inflammation is associated with cachexia, where IL-6, TNF-a, IL-1b, and INF-g may play a major role. Additionally, IL-6 is associated with the control of hunger via its action in the hypothalamus and directly on fat and muscle cells. Therefore, as seen in VL, the systemic inflammation mediated by inflammatory cytokines, especially IL-6, is the primary determinant of wasting.

- Anemia in VL is typically iron deficient, with hypochromia and microcytosis with low sera iron concentration. Hepcidin, high in VL, is an iron-regulating peptide hormone made in the liver, adjusting the release of iron from enterocytes absorbing iron, erythrocyte-recycling macrophages, and iron-storing hepatocytes to plasma. Hepcidin acts by inactivating the cellular iron exporter, ferroportin, which delivers iron to plasma from all iron-transporting cells for hematopoiesis in the bone marrow. Indeed, IL-6, IL-1b, and IL-8 are negatively correlated with anemia in patients with VL. However, the destruction of red cells by the enlarged spleen, e.g.,

hypersplenism certainly contributes to anemia, but the pathogenesis of anemia in VL is mediated mainly by APR secondary to the inflammatory cytokines, especially IL-6.

- Edema, a sign of severe VL, is also a consequence of the APR. Since albumin is decreased as a negative APR and is the main protein responsible for oncotic plasma pressure, hypoalbuminemia is VL's primary determinant of edema. It is essential to highlight that hepatic failure in patients with VL is rare. Indeed, we have observed that IL-6 is much higher in patients with edema.

- Dyspnea has been described as a strong risk factor for death in patients with VL. There is a leishmanial interstitial pneumonitis in patients with the disease in which amastigotes are rare or absent. Detection of antigens in the sputum suggests the *Leishmania* origin, reinforced by computer tomography findings associated with cough and dyspnea. The protracted disease and similarity with acute respiratory distress syndrome suggest that inflammation through systemic inflammatory response may play a central role in dyspnea observed in VL.

- Nephritic and nephrotic syndromes and low-grade renal failure are frequently observed in VL. These conditions are associated with interstitial nephritis and distinct glomerular involvement like collapsing segmental and focal glomerular sclerosis, necrotizing segmental and focal glomerular sclerosis, and membranoproliferative lesion. In summary, the pathogenesis of interstitial nephritis of VL may be the same as that of systemic inflammatory diseases, which are heterogeneous, involving both productions of autoantibodies and deposition of immune complexes within the interstitium and activation of inflammatory pathways. AA amyloid glomerular deposits without mesangial hyperplasia have also been registered, likely as part of the APR. However, it has to be kept in mind that the first suspected cause of interstitial nephritis in VL is drug toxicity since most anti-leishmanial drugs are nephrotoxic.

Regarding the elevation of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) with jaundice by direct bilirubin denotes some

grade of hepatitis. In patients with VL, it has been shown that hepatocytes may show fatty change varying in intensity and mild degenerative changes with either focal and individual necrosis. An association between liver inflammation has been observed for IL-1 and TNF-a in virus hepatitis, suggesting that the same might be true for VL. Our observations found the strongest correlation between AST and IL-6 and IL-8. However, only IL-6 was associated with AST in a multivariate linear regression. Inflammatory cytokines also mediate the common hyperglobulinemia seen in patients with VL. B-cell differentiates into antibody-producing plasma cells under the influence of cytokines such as IL-6. These polyclonal plasma cells increase in the bone marrow, gut, lymph nodes, spleen, and liver. They produce electrophoretically heterogeneous, chiefly the gamma globulin band. Therefore, the polyclonal increase in the immunoglobulin of VL reflects the expansion and differentiation of plasma cells mediated by IL-6.

- Vomits are associated with increased mortality of patients with VL. At uni- and multivariate analysis, it has been found that IL-6 is associated with this sign, which seems to be a marker of severe systemic inflammation. Although diarrhea is a prevalent symptom of VL caused by *L. infantum* and parasites are seen in the intestinal mucosa, no association of diarrhea with any marker of disease pathogenesis has been found.

- Post-VL dermal leishmaniasis (PKDL) is caused only by *L. donovani* in immunocompetent patients. Infection by *L. infantum* causing PKDL occurs only in patients with AIDS. Therefore, parasite genetics might partially explain the disease. Lesions of the South Asian type are papulonodular, polymorphic, occurring in 5-10% of the VL patients 2-3 years after treatment. They rarely cure spontaneously and have a high parasite burden with dense and diffuse infiltrate. The East African type is macular, with monomorphic lesions, occurring in 50-60% of the patients after treatment or during the treatment but 85% self-heal. Lower parasite burden is noticed, and the inflammatory infiltrate is scarce and patchy. It has been proposed that PKDL happens when the specific immunity is reactivated after cure leading to a decrease in regulatory T cells, TGF-b and IL-10 levels, and an increase in IFN-g, TNF-a, and IL-12. *L. donovani* persisting in the skin is detected by reactivated immune cells, which infiltrate into the cutaneous

tissue causing dermal inflammation by the secretion of IFN-g, giving rise to the dermal manifestations.

- Amyloidosis has been described in patients with VL before specific treatment became available, but a few cases of HIV-infected patients develop renal failure due to amyloidosis with elevated levels of serum amyloid. Indeed, serum amyloid is one of the positive APRs. Amyloidosis also has been described in dogs and hamsters with VL.

- Pancytopenia (reduced red, white blood cells, and platelets) is typical of VL but also occurs in the enlarged spleen by non-inflammatory or infectious diseases, in a process called hypersplenism as seen in the pre-hepatic portal hypertension caused by schistosomiasis. However, in VL patients, the cytopenias are more severe and may depend more on the local spleen inflammation environment, exacerbating the mechanical destruction of blood cells and platelets due to splenomegaly. Indeed, splenectomy of patients with VL completely recovers cytopenias. As discussed above, anemia is mainly a consequence of APR via hepcidin. Comparing the bone marrow cellular population with peripheral blood counts revealed that severe anemia in VL has erythroid hyperplasia and dysplasia instead of just hypoplasia, denoting the mixed effects of red cells spleen destruction and iron deficiency. The prominent white cells alteration in VL is neutropenia, determining leukopenia. Bone marrow myeloid lineage of patients with VL and severe neutropenia is unrelated to bone marrow cellularity indicating that neutropenia is not caused by local changes but, instead, to hypersplenism and to systemic pathogenetic mechanisms.

With or without bleeding, sustained disseminated intravascular coagulation (DIC) has been consistently described in VL. Consumption of coagulation factors, including platelets, is characteristic. Since bone marrow megakaryocyte cellularity is not associated with blood platelet count in patients with VL, thrombocytopenia, commonly seen with the other two cytopenias, is determined mainly by DIC. However, non-inflammatory hypersplenism may also be acting. Correlation studies of thrombocytopenia with DIC and spleen size may clarify this hypothesis. The leading cause of death in patients with VL are Gram-negative and Gram-positive bacterial

infections. We have observed that patients who die from bacterial infections do not have simultaneous hemorrhages, suggesting that both phenomena follow distinct pathways (albeit bacterial infections are also important causes of DIC and hemorrhages). Since patients with VL have high plasma concentrations of IL-10 concomitant to pro-inflammatory cytokines, it may be speculated that immunoparalysis, as seen in septic patients, might be involved in the high risk of bacterial infections of patients with VL. Patients with VL and bleeding phenomena have higher DIC markers and low platelet counts, indicating the primary pathogenetic mechanism for hemorrhages and the consequential death of patients with the disease. DIC in VL is correlated with inflammatory cytokines such as INF-g, IL-6, and IL-8. However, the complementary role of other phenomena cannot be ruled out, such as direct bilirubin retention or mild impairment of liver function. Indeed, bilirubin is essential for vitamin K-dependent coagulation factors, and a slight reduction of liver function in producing these factors may amplify the effects of the consumption of these factors by DIC. Addressing DIC and fibrinolysis might improve the prognosis of patients with severe VL. Although bacterial infections and hemorrhages are the leading cause of mortality by VL, we have recently demonstrated that the *L. infantum* genome contributed to the mortality. However, no specific genetic factor has been identified yet, due to the small sample size of patients' clinical data and parasite genomes. Due to this complex pathogenesis, there is still a long way to go to reduce mortality led by VL.

S7. INNOVATION IN R&D TO CONTRIBUTE TO VL ELIMINATION

S7-01: FUTURE ORAL TREATMENTS FOR VISCERAL LEISHMANIASIS: LXE408, FROM DISCOVERY TO FIRST STUDY IN PATIENTS

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Visceral Leishmaniasis (VL), also known as kala-azar, is a vector-borne disease transmitted by sandflies. It is one of the most neglected tropical diseases and is found in South Asia, East Africa, Latin America, and the Mediterranean. It is caused by the protozoan parasite *Leishmania donovani,* with the main vector being *Phlebotomus argentipes*. The disease is characterized by insidious presentation of splenomegaly, irregular fevers, anaemia, pancytopaenia, weight loss, and weakness, occurring progressively over a period of weeks or even months, leading to death if untreated. An estimated 50,000 to 90,000 new cases of VL occur worldwide annually with children below the age of 15 years representing half of the VL patients affected. Therapies to treat VL include pentavalent antimonials, amphotericin B deoxycholate, liposomal amphotericin B, miltefosine, and paromomycin have been developed and registered in various countries for the treatment of VL. Combinations of these treatments have demonstrated safety and efficacy only in some settings. However, these drugs still have important limitations such as parenteral administration, potential teratogenicity, hepatotoxicity, and nephrotoxicity, and anaphylaxis, among others. LXE408 is a novel oral pan-kinetoplastid proteasome inhibitor that is being developed in partnership with DNDi for the treatment of adults and children with VL. This first-in-class parasite-selective inhibitor of the kinetoplastid proteasome has potent and uniform anti-parasitic activity

against all kinetoplastid parasites, including *Leishmania* species causing visceral leishmaniasis (*L. donovani and L. infantum*), as well as parasites causing Chagas disease (*T. cruzi*) and human African trypanosomiasis (*T. brucei*). The antileishmanial activity of LXE408 has been demonstrated in non-clinical mouse models and in a macrophage infection assay against three strains of viscerotropic *Leishmania* species. LXE408 exhibits a dose-dependent reduction in the liver parasite burden after 8 days of dosing in mice. The pharmacokinetics and clinical safety of LXE408 were characterized in a first-in human, randomized, subject blinded, placebo controlled, single and multiple ascending doses. LXE408 demonstrated safety, tolerability, and favorable PK properties to advance to clinical development studies in patients with VL.

S7-02: BRINGING THE TREATMENT CLOSER TO THE COMMUNITIES: TEST AND TREAT APPROACH BY COMBINING A POINT OF CARE DIAGNOSTICS WITH THE NEW ORAL SAFE DRUGS

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Efforts to control visceral leishmaniasis (VL) in South East Asia (SEA) have contributed to an important decrease in the global number of VL cases, but other regions such as Brazil and eastern Africa remain as large foci. Targets for VL in the Road Map for Neglected Tropical Diseases (NTDs), 2021-2030 include: i) advance elimination of VL as a public health problem in up to 85% of endemic countries and validate elimination in SEA; and ii) that in countries where post kala azar dermal leishmaniasis occurs 100% of cases are detected and treated. Diagnostics are key to achieve these targets, as early diagnosis (both laboratory and clinical) is one of the core interventions for VL. But the current diagnostic tests and strategies are not enough to reach these targets. Microscopy is still the reference test for VL diagnosis with all its inconveniences, i.e. requires access to some equipment and trained personnel plus need for invasive sampling (bone marrow or spleen aspirates) to achieve good sensitivity. Antibody-detection tests, mainly rapid tests (RDTs) and direct agglutination tests, are also part of the VL diagnostic algorithm, despite not being markers of active infection. Different nucleic acid amplification tests have very good diagnostic performance using a less invasive sample, such as peripheral blood, but despite the fact that they have been used for about two decades in reference centres, the barriers to their implementation in less-resourced settings, where they are needed most, have not been addressed. There are a number of actions required to eliminate VL as a public health problem, and to some extent these are linked to the existence of improved or new diagnostics and diagnostic strategies. More specifically: i) to develop more effective and user-friendly diagnostics, especially for eastern Africa, ii) devise less-

invasive and highly specific tests to measure parasite level, iii) develop less invasive test of cure for PKDL and VL, and iv) design and apply strategies and tools for patient tracking. Other critical actions highlighted in the Road Map for NTDs, and that relate to improved diagnostics, include: i) understanding of parasitic and patient factors linked to a fatal prognosis, ii) develop an improved leishmanin skin test, iii) enable early detection to ensure prompt treatment, through, for example, active case detection, reducing time between onset of symptoms and treatment, and iv) improve deployment of trained health personnel at community level and maintain awareness within health systems and community to ensure detection and treatment of cases. New and improved diagnostic tools and strategies will ensure access to diagnosis at the primary health centres, where is urgently required. And, with new fully oral drug therapies to come, will allow a more decentralized management and the implementation of a test-and-treat approach. Moving towards an elimination/near elimination context, the new tests should enable diagnosis strategies for active case finding and treatment. These new tests should also address current threats such as the discontinuation of production of the antibody detection RDT that best performs in eastern Africa (IT-Leish).

S7-03: PKDL TREATMENT – WHY IT IS IMPORTANT FOR VL CONTROL AND ELIMINATION, AND THE NEED FOR IMPROVED TREATMENTS

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ln 2005, the governments of Bangladesh, India and Nepal launched a Kala-azar Elimination Programme (KAEP). There were several factors favourable for the elimination of kala-azar in this region. The key strategies for elimination were early diagnosis, efficacious treatment and vector control. The KAEP results has been very encouraging with most participating countries either have attained the target or close to attaining it. Patients with post kala-azar dermal leishmaniasis (PKDL) are a reservoir for the parasite, therefore these patients are a major threat to elimination or start of a fresh epidemic of visceral leishmaniasis (VL). Outbreaks of VL have been attributed to PKDL patients. There are reports which support this notion. These patients are highly infectious to sand flies, nodular lesions more likely and macular lesions are less likely to infect the sand flies. Early detection and effective treatment of PKDL are important for VL elimination. As patients are asymptomatic they do not come forward for treatment and the non-compliance and lost to follow-up are common occurrences. Currently recommended regimen for PKDL treatment (12 weeks of miltefosine) is too long, resulting in frequent non-adherence. Further, serious ocular toxicities attributed to this miltefosine regimen, call for the development of new and short treatments for PKDL. There should be no complacency once KAEP target is achieved, and it is important that we detect PKDL cases early in community by active case detection. These patients should be treated with safe and effective regimens. Unfortunately, at present no PKDL treatment regimen is completely safe and efficacious. If we have to sustain the elimination effort, we have to combat this dangerous menace of PKDL. Thus we urgently need short, safe and effective treatments for PKDL.

S8. SAND FLY SALIVA AND IMMUNE RESPONSE OF BITTEN HOSTS

S8-01: IMMUNE RESPONSE TO SALIVARY LinB13 AND DISEASE SEVERITY IN TEGUMENTARY LEISHMANIASIS

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We have previously shown that seropositivity to rLinB-13, a salivary protein from *Lutzomyia intermedia*, predicted sand fly exposure and was associated with increased risk of developing cutaneous leishmaniasis (CL). Herein, we investigated the cellular immune response to saliva from *Lu. intermedia*, using rLinB-13 as a surrogate antigen in naturally exposed individuals presenting positive serology to LinB-13. We also investigated the response to rLinB-13 in leishmaniasis patients, displaying active ulcers and positive PCR for *L. braziliensis*. Peripheral blood mononuclear cells (PBMCs) stimulated in vitro with rLinB-13 secreted elevated levels of IL-10, IL-4, IL-1β, IL-1α, IL-6 and chemokines (CCL3, CCL4, CCL5 and CXCL5). CL, and disseminated leishmaniasis (DL) patients displayed a significantly higher IgG response to rLinB-13, compared to healthy subjects and anti-rLinB-13 IgG was positively correlated with the number of lesions in DL patients.

Positive serology to rLinB-13 was also associated with chemotherapy failure. PBMCs from DL patients stimulated with rLINB-13 secreted significantly higher levels IL-10 and IL-1β compared to CL individuals. In this study, we observed an association between humoral and cellular immune response to the sand fly salivary protein rLinB-13, and disease severity in tegumentary leishmaniasis. This study brings evidence that immunity to rLinB-13 influences disease outcome in *L. braziliensis* infection and results indicate that positive serology to rLinB-13 IgG can be employed as marker of DL, an emerging and severe form of disease caused by *L. braziliensis*.

S8-02: ANTI-SALIVA ANTIBODY PRODUCTION IN NAIVE DOGS EXPOSED TO UNINFECTED *Lutzomyia longipalpis* BITES

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Canine visceral leishmaniasis (CVL) is caused by *Leishmania infantum* and transmitted to dogs and humans by sandflies. In Brazil, *Lutzomyia longipalpis* is the primary vector of this disease. When feeding, infected sandflies inoculate metacyclic promastigote forms of *Leishmania* and their saliva and other components into the hosts. Anti-saliva antibodies were associated with increased visceral leishmaniasis severity in naturally infected dogs. Although these compounds introduced by the vector favor the establishment of *Leishmania*, the early events that occur at the bite site are not fully understood. A better understanding of these initial events is essential to the development of better therapeutics and prophylactic strategies. Studies have demonstrated that sandfly saliva promotes *Leishmania* infection. *Leishmania major* co-injected with *Lu. longipalpis* or *Phlebotomus papatasi* saliva resulted in more severe disease manifestations in mice, as reflected by larger lesions compared to animals that received only parasites. This initial observation was further supported by additional studies demonstrating the enhanced infectivity of *L. major* when co-inoculated with saliva from the sandfly *Lu. longipalpis*. Apart from antihemostatic properties, sand fly saliva promotes chemotactic activity in a variety of immune cells, such as macrophages, neutrophils, dendritic cells and lymphocytes. In addition, many other cell types, including monocytes, interact with sandfly saliva, thereby modifying inflammatory processes at

the blood feeding site. It has been proposed that the resulting effects on the host immune system contribute to increased parasite loads in mice exposed to sandfly bites compared to animals infected through needle injection7. Moreover, it has also been demonstrated that other vector-derived factors can additionally contribute to *Leishmania* infection, such as the microbiota of the vector, exosomes and the promastigote secretory gel. In recent years, our group has contributed compelling data linking the differential production of lipid mediators to inflammatory factors involved in the establishment of infection. Specific levels of lipid mediators, mainly the eicosanoids leukotriene B4 (LTB4) and prostaglandin E2 (PGE2), are important components of the inflammatory response to, and outcome of infection by intracellular pathogens. Previous in vitro studies have demonstrated the role of LTB4 as a factor that participates in parasite killing, while PGE2 was shown to favor *Leishmania* survival. More recently, lipid mediators were identified as biomarkers of cutaneous and visceral leishmaniasis severity. Anti-sandfly saliva antibodies could also represent an essential epidemiological tool to assess vector exposure in endemic areas. LJM11 and LJM17 recombinant proteins are present in the vector's saliva and have already been used for this purpose. Our goal was to follow up anti-saliva antibodies (anti-LJM11 and anti-LJM17) production in naïve dogs experimentally exposed to *Lu. longipalpis* sandflies. We also assessed the persistence of anti-saliva antibodies titers for one year, and after re-exposure to the sandfly vectors. Blood samples from the dogs were collected weekly to assess the production of anti-LJM11 and anti-LJM17 IgG by ELISA. Six healthy naïve dogs were exposed weekly to 35 *Lu. longipalpis* female sandflies until at least 80% of the female were fed. Dogs were exposed to the sandflies until anti-saliva antibody production reached a plateau and remained elevated for at least three consecutive weeks. Afterward, we ceased sandflies exposures; we followed the dogs weekly until the animals tested negative for anti-saliva antibodies for three consecutive weeks. Then, we re-exposed the dogs to the sandflies and evaluated the time-period it took for the animals to resume anti-saliva antibody production. The Reactivity Index (RI) was calculated by dividing the optical density by the cut-off point obtained in each ELISA plate to compare antibody production. Soon after the first exposures, there was an immediate increase in the production of anti-saliva antibodies (between the first and the third week).

On the twenty-eighth day after the first exposure (with a median of 10.5 days), all six animals showed detectable anti-saliva IgG titers. Dogs were exposed to sandflies for six to nine weeks (with a median of 52.5 days). After the initial rising of anti-saliva antibody production post-exposure, anti-saliva antibody titers fluctuated, remaining detectable for over a year. We found a statistically significant difference comparing anti-saliva antibodies titers before exposure and five weeks after the exposure ($p<0,05$). Despite the variations in titration, four dogs remained positive for 41 weeks (290 days) on average, two animals are still positive after 460 days. After the first week of re-exposure, dogs demonstrated antibody titers rising significantly. Throughout the evaluation, there was a considerable variation in antibody production among the six animals, especially concerning the time of seroconversion, time to reach the plateau, and titer decay. Although we observed differences among the animals, we can detect a similar pattern during the follow-up. Currently, studies evaluating the cellular immune response of these animals are being carried out. We have collected peripheral blood mononuclear cells (PBMC) in different time points after exposure and re-exposure and we intend to stimulate these cells with salivary gland homogenate and measure the cytokines production with LUMINEX specific canine kit. Moreover, we will measure canine serum cytokines produced during the follow-up after exposure and re-exposure to sand flies. This experimental approach allows us to better understand the early events among vector and host after exposure to sand flies and to delineate better strategies to control infection establishment

Keywords SANDFLY; SALIVA; ANTIBODIES; RESERVOIR

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S8-03: IMMUNE PATHWAYS OVERREPRESENTED IN THE HUMAN SKIN TRIGGERED BY BITES OF SAND FLY VECTOR ARTHROPODS

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Arthropod vectors such as mosquitos and sand flies deliver infectious pathogens into the skin of humans while taking a blood meal. Pathogens are co-delivered with vector saliva containing a complex mixture of immunomodulatory components, including salivary proteins and microbiota. In animal models, vector bites and saliva are critical for establishing infection and exacerbating disease pathogenesis. However, the mechanisms by which vector bites do this in humans remain poorly understood. To address this, we conducted the BITE Study, the first clinical study to perform global profiling of the human skin immune response to vector bites. Healthy human volunteers were exposed to uninfected bites of one of three vectors of global health importance – the mosquitos Aedes aegypti and Anopheles gambiae, or the New World sand fly Lutzomyia longipalpis. Skin biopsies were collected from bite sites at 4 hrs and 48 hrs after vector exposure. The skin transcriptome from the bite sites was determined by RNA-seq and compared to unbitten skin as a negative control. Immune pathways overrepresented in the skin highlights the immune nature of the response to insect vectors of diseases.

S8-04: HUMORAL IMMUNE RESPONSES IN PEOPLE TO BITES OF OLD WORLD SAND FLIES

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The tiny amount of sand fly saliva associated with bites is immunogenic, with both local and systemic immune responses identified. Our experience with human humoral immune responses to two *Leishmania major* cutaneous leishmaniasis vectors, *Phlebotomus papatasi* and *Phlebotomus duboscqi,* will be presented. Additionally, both humoral and cellular immune responses to *Phlebotomus alexandri* in those with asymptomatic Iraq-acquired visceral leishmaniasis will be discussed. These observations will be placed in the context of other published studies of humoral immunity after Old World sand fly exposure. The role of human antibody responses to salivary molecules is of keen interest in the development of biomarkers of vector exposure and as exploration for novel target antigens in future *Leishmania* vaccines.

S9. ELIMINATING VL IN INDIA: THE LAST MILE CHALLENGES AND OPPORTUNITIES

S9-01: ADVOCACY AND COMMUNICATIONS NEEDS TO ENABLE THE NEW REGIONAL VL STRATEGY OUTCOMES – LEARNINGS FROM PROJECTS'

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Sustained political will is vital to ensure evidence results in policy shifts for positive change. India and the South Asian and South-East Asian Region (SA & SEAR) have reached the last mile of VL elimination. Today, community participation and social mobilization at the unit level and inter-sectoral partnerships at the systemic level play a significant role in ensuring effective programme implementation and uptake of services by intended communities. Sustained political commitment at the local and highest levels, elevated through innovative advocacy and strategic communications will ensure India, and the SA & SEAR meet the VL elimination target set by the World Health Organization-published NTD Roadmap 2021-2030. Since 2005, regional collaboration and cross-learning *(such as the introduction of the more effective compression pumps in India, following Nepal and Bangladesh's experience)* has been a focal point of the VL elimination strategy in SA & SEAR. This was understandable, since at the time, more than 50% of the cases emanated from the border districts in the three endemic countries. Regional collaboration has resulted in effective case management *(including the introduction of the more effective single dose Liposomal Amphotericin B)* and vector control *(such as the use of pyrethoid-alpha cypermethrin)* and has helped India and Bangladesh reduce VL incidence by 30 times and Nepal by 9 times. Today, VL elimination is within reach for

India and other countries in the SA & SEAR. However, a shift in the disease landscape has resulted in new challenges. The three endemic countries have witnessed a surge in asymptomatic infections in recent years. In 2020, such infections were almost 9 times the clinical infections in India and Nepal and 4 times the clinical infections recorded in Bangladesh. Thailand and Bhutan have also witnessed sporadic cases. Historically, a reduction in clinical infections has resulted in a roll-back of programmatic efforts. Sustained high-level political commitment, gained through consistent advocacy and communications, is needed to ensure continued prioritization so that VL elimination efforts reach the last mile. Since 2018, partner organizations including PATH, CARE, Clinton Health Access Initiative, World Health Organization, Global Health Strategies, Project Concern International and others have supported the Government of India's efforts to eliminate the disease as a public health problem. Partners support government efforts including early diagnosis and complete treatment, integrated vector management, including indoor residual spraying (IRS), advocacy, communications for behavioral change, and inter-sectoral convergence, surveillance, supervision, monitoring and evaluation, capacity strengthening and programme management. Consistent, deliberate, and innovative evidence-based advocacy with policy and decision makers was implemented to create a conducive political environment for improved awareness, access, and acceptance of available programmatic services in endemic states in India. Sustained outreach and engagement with political leaders across party lines to sensitize and engage them on challenges around VL elimination was ensured to increase interest. Coalition and champion building were leveraged as core techniques to promote leadership and advocacy through policy and decision makers. Voices of technical champions, who played significant roles in regional communities of practice were leveraged through commentaries, media pieces, interviews, high-level meetings etc. Consistent media advocacy was utilized to ensure a steady drumbeat around the need for VL elimination in the public discourse. High-impact, high-visibility campaigns with participation by diverse stakeholders were implemented to not only spotlight remarkable achievements but also bring attention to the need for continued prioritization. Methods have led to increased prioritization, both at the local, state levels, as well as at the national level. Targeted interventions

have resulted in a 78.9% decrease in the number of VL cases *(6,377 cases in 2016; 1,345 cases in 2021).* Further, by October 2021, 99% of the total 633 endemic blocks had achieved the elimination target of less than 1 case per 10,000 population at block level. Between November 2019 - May 2022, over 300 champions *(including political leaders, national and state government representatives, media and civil society representatives, popular influencers, and celebrities)* made more than 650 supportive statements to highlight VL elimination. These included public commitments by two consecutive Union Ministers of Health and Family Welfare, and State Health Ministers of the four endemic states, Members of Parliament, and Member of Legislative Assembly. Leading political parties have highlighted their commitment to VL elimination in their party manifestoes and integrated programme activities within the fold of high-visibility flagship programmes. Despite being consumed by two devastating waves of the COVID-19 pandemic, IRS rounds continued in India. Districts and blocks in states have been rewarded for meeting elimination targets and for sustained surveillance. For instance, the total approved budget for West Bengal increased over 280% from 26.4 million Indian rupees in FY 2019-20 to 102 million Indian rupees in FY 2021-22 as it achieved the elimination target. Partners supported independent assessments and joint monitoring missions to ensure continued surveillance. Awards and recognition have been recommended to improve block-level performance. Free-of-cost treatment is provided to confirmed cases of VL and Post-Kala azar Dermal Leishmaniasis (PKDL), at government hospitals, monitored until treatment is completed, and followed up for 12 months to detect adverse drug reactions, treatment failure and relapse. Kala-azar cases are also followed-up for 3 years to detect PKDL. Evidence generation, evidence synthesis, advocacy and communications are often integrated and continuous. As technical expertise across the region underlines the need for innovation, consistent advocacy, particularly with policy and decision makers is necessary to achieve policy shifts. Local, reliable evidence, synthesized, simplified, and shared with diverse stakeholders including political leaders can generate and maintain interest in eliminating VL. Through consistent advocacy, capacities of aware, willing *'champions'* can be strengthened to use available evidence to communicate for positive change. Such champions can then be encouraged

to mobilize to highlight the need for and raise the priority accorded to VL elimination.

S9-02: TERRAIN - VL IN JHARKHAND

Arun Kumar Singh

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The Kala Azar situation in Jharkhand is somewhat unique. One cluster of four districts in the north and east, the Santhal Pargana area, is the only affected part of the state. The other regions and districts have not yielded cases passively or when health workers looked for them, though the geographical, social, and economic characteristics are almost similar. Other diseases, including vector borne ones like malaria and lymphatic filariasis, however, affect all regions of the state. The state had the highest annual incidence rate of VL in a tribal region that is sparsely populated, forested and hilly. Indoor Residual Spraying, the mainstay of prevention, was implemented in the state with local adaptations to suit the cultural context and the challenging terrain. It was closely monitored, with accurate coverage estimates independently after each round, to reach over 90% of targeted households, at which it is maintained since several years.

Introduction and revamping of the honorariums and other financial incentives to the patients, health workers, spray workers, volunteers and other involved people went a long way in improving not only the spray work, but active case finding, treatment completion and the conduction of the program in general. Special care is taken to ensure that payments remain up to date. Careful monitoring of the situation in every village and health center and ensuring supply of logistics and medicines have been built up. Since every Primary Health Center in the affected area is a treatment center, ensuring training to the medical and paramedical staff is high on the agenda. Correct entry in the KA MIS, through regular oversight ensures that the program data is available at every level for analysis and data driven program

decision making, thus program management. The presentation will include an analysis of program efforts, challenges, results and lessons.

Keywords VL ELIMINATIONS, JHARKHAND – INDIA, KA ELIMINATION, JHARKHAND– INDIA, KALA AZAR, JHARKHAND

S9-03: HOW BIHAR BROUGHT KALA AZAR TO THE THRESHOLD OF ELIMINATION

Sanjay Kumar Singh

Government of Bihar

Ninety-eight million people living in 458 blocks of 33 districts of Bihar are at risk from Kala Azar, by far the widest of any state in India, historically. While Bihar accounted for 67% of India's cases in 2011, when the incidence of the disease was at its latest peak, and in 2021 it accounts for about 72% of India's disease burden. A total of 967 cases were registered in 2021. This is the eleventh consecutive year of decline since the last peak of 25,222 cases reported in 2011 and a 97% reduction in the annual incidence of Kala Azar since the previous major peak in 2007, when 37,822 people were reported to have had the disease. As of the end of last year 2021, only 2 blocks (sub-districts) remain above the elimination threshold. Along with the major burden of the disease, the state has borne the major responsibility for its elimination as well. It has done so by making sure that the main interventions for preventing the spread of the disease reach all villages and families at risk and all patients are detected and treated at the earliest. The state ensures that all villages at risk receive Indoor Residual Spray (IRS), a massive operation that is managed entirely by the state, using Synthetic Pyrethroid insecticide supplied by the Government of India. As estimated independently, IRS now reaches around 85% of all targeted households every round, and almost 90% of all rooms used to sleep in are sprayed. Administrative decision of increasing the amount of the wages and ensuring timely payment of spray squad seems to have worked along with proper planning of the operations of IRS. Almost all cases of Kala Azar in the state are diagnosed and treated in government hospitals, using highly effective medications, fully free of cost. This is made possible by ensuring that hospitals are well-equipped and supplied, and staff are trained. Each case of Kala Azar is paid a substantial amount of Rs. 6600 from the Chief Minister's fund and Rs. 500 from NHM head as compensation for wage loss due to the

illness. Every case is followed up after diagnosis for at least three years to ensure that there is no relapse and that the occurrence of PKDL is picked up as soon as possible. The dedicated information system, KA MIS, is robust and gives confidence that program managers are in full control of all aspects of the elimination program.The progress towards Kala Azar elimination is closely monitored at the highest level. The presentation will cover the strategic and operational challenges and lessons that were learnt on the path to bringing the disease down to a level where it is no longer a public health problem.

Keywords IRS; VL ELIMINATIONS; BIHAR – INDIA; KA ELIMINATION; BIHAR– INDIA; KALA AZAR; DISEASE

S9-04: THE LAST MILE CHALLENGES FOR ELIMINATION OF KALA-AZAR IN WEST BENGAL, INDIA, 2022

Tushar Acharyya

Government of West Bengal

Most people are aware of the connection between malaria and Kolkata, that the parasite was discovered by Major (later Sir) Ronald Ross at a hospital in the city. What many not be aware is that one the scientists who discovered the parasite that causes Kala Azar, Sir William Leishman, worked in Kolkata as well. This tradition was carried forward by Dr U N Brahmachari, an Indian scientist of the city who put forward the first effective treatment for this disease, Urea Stibamine, a few years after the parasite was discovery. After the disease saw a steep decline in the 1950s and 60s, the resurgence from the 1970s was mainly in the states of Bihar and Jharkhand. During this time in Bengal, this time, was spared the dubious distinction of being the epicenter of this disease. However, it took careful implementation of public health measures to rid all the blocks of disease to the extent that all blocks now have incidence rates below the level stipulated for Elimination of Kala Azar as a public health problem. This happened about four years ago. Being the first state in the country to bring about Elimination of Kala Azar, the program moved to implement the post elimination strategy, which is continuing surveillance in the areas where the disease was rampant in the past. These areas like Phansidewa in Darjeeling district and Habibpur in Malda are near to the borders with Nepal, Bangladesh and Bihar, all endemic areas. This is a challenge. Special efforts have been and are being made to detect and treat PKDL cases, known to be the reservoir of the disease. The attention now is to look at the atypical presentations which may occur due to concurrent infections with tuberculosis and HIV. In fact, all cases of PKDL are tested for HIV and TB, and cases of these diseases are checked for PKDL. In the future the program in the state will be interested to see if alternate therapies for VL and PKDL become available in view of the increasing numbers of coinfection of both with TB and HIV.

Keywords VL ELIMINATIONS; WEST BENGAL – INDIA; KA ELIMINATION – WEST BENGAL; KALA AZAR IN WEST BENGAL; INDIA

S10. NEW TRENDS IN THE DIAGNOSIS OF CHAGAS DISEASE

S10-01: UTILITY OF CELLULAR IMMUNE MARKERS IN CHRONIC CHAGASIC HEART DISEASE

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One of the most intriguing question during chronic Chagas heart disease, caused by *Trypanosoma cruzi*, is the transition from a chronic asymptomatic infection to a symptomatic one. The pathogenesis of Chagas disease has been associated with various factors such as the parasite genotype, the clinical phase of the disease and the immune response. For more than 15 years, our working group has studied the immune response in patients with chronic Chagasic heart disease. These studies have been done comparing chronic asymptomatic (undetermined) patients, chronic symptomatic (determinate) patients with healthy individuals and individuals with non-infectious chronic heart disease, and occasionally with chronic chagasic patients with heart transplant. Experiments have been carried out mainly with peripheral blood mononuclear cells, and some with cells extracted from cardiac explants of transplanted patients. The results indicate that T lymphocytes from patients with Chagasic cardiac disease present alterations in proliferation and a lower frequency of total CD8+ TSCM cells; they also have a lower number of central memory antigen-specific CD8+ T cells and a higher number of antigen-specific CD8+ T cells with late differentiation, and a marked expression of inhibitory receptors (CD160,

PD-1 and CTLA4). One of the markers of functionality of lymphocytes is their ability to secrete cytokines after antigenic stimulation. After evaluating the production of IL-2, TNF-α and IFN-γ by the same cell (multifunctionality), it was shown that as the severity of the disease progresses, the frequency of multifunctional memory CD8+ T lymphocytes decreases, characterizing the most advanced phase of the disease by the presence of monofunctional cells. These findings indicate the existence of an adaptive cellular immune response mediated by CD8+ T cells with the signature of clonal exhaustion. Using all these cellular markers should be possible to define the progression of chronic Chagas diseases.

S10-02: POINT-OF-CARE MOLECULAR DIAGNOSIS OF CHAGAS DISEASE: FROM FIELD TO LABORATORY

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Due to its neglect, enhancing access to diagnosis of patients at risk of suffering *Trypanosoma cruzi* infection should be a priority at national and regional levels. However, there is no consensus yet on the diagnostic algorithms for many scenarios of *Trypanosoma cruzi* infection, which difficult the establishment of public health guidelines in endemic and non-endemic countries. One of those scenarios that need improvement in terms of diagnosis is mother-fetal transmission of *T. cruzi, which* causes congenital Chagas disease, a re-emerging infectious disease that affects endemic and non-endemic regions alike. An early diagnosis is crucial because its prompt treatment achieves a high cure rate, precluding evolution to symptomatic chronic Chagas disease. However, early diagnosis involves low sensitive parasitological assays, making necessary serological confirmation after nine months of life. In such situation a big proportion of infants, specially from rural areas, is lost to follow-up due to economical constrains. Molecular methods in newborns or infants have been tested for sensitive early diagnosis and bypass loss to follow-up. A TaqMan real-time polymerase chain reaction (qPCR) based kit including an internal control of DNA integrity of the sample or reaction inhibition, has achieved higher sensitivity than the micromethod starting from 1 mL of peripheral blood. Several studies in infants born to seropositive mothers have observed that the best age to carry out molecular diagnosis of congenital transmission is around the first month of life, when the parasitic load is at its peak and potential false positive results that might arise from the contamination with *T. cruzi* DNA from the mother to the fetus are minimised. Loop mediated isothermal amplification (LAMP), is an alternative molecular approach, suitable to resource-limited laboratories, because the strand-displacement-*Bst* DNA polymerase works at 60-65ºC and does not require of a thermocycler, but

only a thermoblock or water bath. Furthermore, product visualisation can be done by the naked eye or followed in real time by turbidity or fluorescence. LAMP procedures have been proposed for detection of *T. cruzi* infection. There are a few in-house LAMP methods for *T. cruzi* and a prototype kit based on *the parasite* satellite DNA sequences and containing dried reagents on the inside of the microtube caps (Eiken Chemical Co, Japan).

Aiming at implementing early diagnostic strategies suitable for minimally equipped laboratories associated to Maternities in endemic countries, this *T. cruzi*-LAMP kit was coupled to different rapid DNA extraction methods and supports.

1) an automated DNA extraction device re-purposed from a 3D printer (PrintrLab extraction device, AI Biosciences, Inc., College Station, Texas, USA). DNA is purified using a Multi Sample DNA extraction kit based on magnetic beads. The procedure has been optimized for 200 µL of starting blood anticoagulated with EDTA. The whole process takes less than three hours to yield a result. A recent pilot study in Yacuiba, Bolivia, showed that this 3D printerLab-LAMP duo strategy was able to detect congenitally infected neonates who were not identified by means of the current parasitological method and showed high agreement with PCR.
2) an ultra-rapid DNA extraction method (PURE, Procedure for Ultra Rapid Extraction- Eiken Chemical Co, Japan) that uses only 30 uls of starting blood anticoagulated with heparin and the DNA is obtained in around 10-15 minutes. It has three components, a heating tube that contains Sodium hydroxide for sample lysis, an adsorbent tube that contains an adsorbent powder that eliminates proteins and other potential inhibitors, and injection cap that contains the membrane that allows the elution of the single stranded DNA, while the debris is retained. This method has been analytically validated in artificial blood samples containing serial dilutions of *T. cruzi* cultured parasites from different discrete typing units.

The next challenge will be to test dried blood spots using Flinders Technology Associates (FTA®) cards to expand the possibility of rapid LAMP diagnosis of Congenital infection to those babies born in domiciles or

rural areas without a laboratory suitable for LAMP procedures. These cards consist of filter paper impregnated with a proprietary chemical mixture that lyses cells, inhibits overgrowth of bacteria and other microorganisms, denatures proteins and immobilises nucleic acids in a matrix, designed for long-term storage at room temperature. In the context of vector-borne diseases, FTA cards have been used for successful preservation of different protozoan pathogens, including *Plasmodium falciparum*, *P. vivax* and *P. berghei* and *Trypanosoma brucei,* but very few data exist regarding their feasibility for detection of *T. cruzi have been reported.* These procedures are being actually under a process of transference to laboratories linked to Maternities in endemic sites of Argentina, Bolivia and Paraguay (Project ChagasLAMP Project (ref.: G2020-203) from the Global Health Innovative Technology Fund and a small grant program of PAHO/WHO/TDR (LEG ID39002). Monitoring of parasitological response to treatment by means of surrogate markers may guarantee more accurate follow-up and earlier detection of treatment failure. However, we still lack sensitive surrogate markers of treatment failure that can be applied with simple laboratory manipulations and inexpensive equipment. Up to date, no studies have tested LAMP, as surrogate means for monitoring response to anti-parasitic treatment. This would be worthwhile because most patients live in resource-limited settings with poor investment in public health policies. We have evaluated LAMP to analyse a series of archival clinical samples collected from Chagas disease patients who received benznidazole to assess its ability to detect *T. cruzi* DNA, which indicates treatment failure, and compared it with standardized qPCR, which is currently used in clinical trials and in clinical practice. In this case, the PrintrLab machine was compared to a manual DNA extraction using silice-columns in 23 paired samples. The agreement between both LAMP procedures was 0.704 [0.39 to 1.00]. The mean difference for both Tc-LAMP data groups compared with the comparator qPCR assay was 0.02 [CI= -0.58-0.62] and -0.04 [CI = -0.45-0.37] respectively, demonstrating the high concordance of the diagnostic methods evaluated (Bland-Altman analysis). LAMP performed well in DNA samples obtained in different supports and stabilizing agents, such as frozen blood treated with EDTA or Guanidine Hydrochloride buffer, as well as in Cerebrospinal fluid specimens from Chagas-AIDS patients. Tc-LAMP detected samples with more than 0.5 par.eq/mL, which is the approximate

limit of detection of the comparator standardized qPCR test (0.69 par.eq/mL for Tc VI CL-Brener clone). These preliminary findings have promoted the design of new prospective studies to validate LAMP in the field for early diagnosis of Congenital Chagas disease and assessment of treatment response using point-of-care DNA extraction and amplification techniques, that may expand the implementation of molecular diagnostics and monitoring of CD to low-resource settings in endemic areas. Beyond PCR and LAMP, other amplification technologies are being investigated, such as recombinase polymerase amplification (RPA). This isothermal reaction needs lower amplification temperature and shorter amplification times than LAMP, plus it has exhibited good performance in comparison to PCR in samples from domestic reservoirs in México. Its combination to a modified 3D-printer low-cost DNA isolation system has been already explored, so it would be worthwhile to evaluate this approach for human *T. cruzi* diagnosis purposes.

S10-03: CHAGAS DISEASE: ADVANCES IN DIAGNOSIS

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The diagnosis of Chagas disease must be clinical, epidemiological and laboratorial. The latter depends on the phase of the infection: if acute, parasitological methods should preferably be used, while if a chronic phase is suspected, indirect serological methods should be used. During the 60-day duration of the acute phase, the classic wet smear and concentration methods, either by Strout or microhematocrit, are the most cost-effective, but require staff training. These tests are most effective within the first 4 weeks of symptom onset. Giemsa-stained smears are usually positive only in high parasitemias, seen in transfusion transmission or immunosuppression. The thick smear, used to diagnose malaria, is useful in cases of fever where plasmodia are not found, but malaria technicians must also be trained for it. In the suspicion of congenital transmission, the mother should be tested by serology. This transmission is rare (5%), and may occur during childbirth, but in some countries has been the main source of new cases. Parasitological tests, including PCR are mandatory, and if negative, a conventional serology in newborns before one year of age is necessary. In all the above conditions, the parasitological diagnosis is important because if confirmed, specific treatment should be indicated, which is effective in the vast majority of cases. Parasitological tests for parasite multiplication (blood culture, xenodiagnosis, inoculation in susceptible animals, PCR) do not ensure that it is an acute phase (because some chronic ones are also positive), they are not readily available, they have high costs and their result is delayed in general. IgM by immunofluorescence is also not recommended due to the lack of kits, because it does not always give positive results and because some chronic patients (15-20%) also have antibodies of this class. If the suspicion is of a chronic phase (the vast majority) that usually presents

with low parasitemia, we must resort to the search for anti-*Trypanosoma cruzi* antibodies, which is achieved with serological methods, which can be divided into conventional and non-conventional. Among the first, of choice, are the immunoenzymatic techniques (ELISA), indirect immunofluorescence (IIF) and indirect hemagglutination. For all of them there are good commercial kits, at affordable prices and easy to use, with technical experience accumulated over more than 4 decades in Latin America. A reliable result depends on good quality kits (which have to be tested on each batch) and good technical training. Staff turnover in many public services makes it difficult to meet these requirements. It is important to consider the different circumstances in which a serological test is requested: the best known is the diagnosis of Chagas or its exclusion, for which the World Health Organization recommends that two tests with different principles be used (ELISA+IFI for example), thus avoiding errors. Under these conditions, it is possible to diagnose (or exclude infection) in more than 98% of cases. The rare cases of inconclusive results are due to cross-reactions with other infections, particularly leishmaniasis. But there are other circumstances in which the serological diagnosis is made, such as in the exclusion of blood donors. In this case, and for operational reasons, a single highly sensitive test, such as ELISA, is sufficient. Serological diagnosis is also used in field surveys, treatment monitoring and transplants where both the donor and the recipient must be studied. Within the unconventional serological tests, there are several types: rapid test, by immunochromatography, which has its indications in emergency situations or in areas of difficult access and epidemiological studies; ELISA with recombinants that has been well accepted; chemiluminescence that uses recombinant antigens and synthetic peptides, used today in hemotherapy services with great demand; western blot (Tesa-blot®) that is no longer commercialized, and other non-commercial ones that are used in research. Most of them are more complex to execute, have a higher cost and are unnecessary in the routine of a diagnostic laboratory. Regarding the use of parasitological tests in the chronic phase, they are restricted to very particular situations, especially in the follow-up of patients treated with chemotherapy (for example, benznidazole), where they are only valuable when they are positive, indicating therapeutic failure. In summary, the laboratory diagnosis in those infected with *T. cruzi* in the chronic phase

should preferably be done with two conventional serological tests, with which it is possible to make an accurate diagnosis in more than 98% of the cases. The use of other tests implies greater complexity and an increase in false positives due to lack of technical instructions that increase the gray region, particularly with chemiluminescence and recombinant ELISA, a topic that we will illustrate in the presentation. Donor exclusion can be done by a single recombinant ELISA or chemiluminescence test. The latter are not useful in the post-therapeutic follow-up, as they remain reactive in general, when the conventional ones have already been negative.

S11. NEW INSIGHTS IN POSTTRANSCRIPTIONAL REGULATION IN *Leishmania*: IMPLICATIONS IN THE PARASITE DEVELOPMENT AND DISEASE CONTROL

S11-01: A CENTRAL MECHANISM REGULATING mRNA ABUNDANCE THROUGHOUT THE *Leishmania* DEVELOPMENT

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The human parasitic pathogen *Leishmania* exhibits unusual features in regulating gene expression and relies entirely on posttranscriptional regulation (PTR) for monitoring mRNA abundance and translation rates throughout its development. It is our hypothesis that this uniqueness is amenable to therapeutic interventions. We have reported previously that more than 20% of *Leishmania* transcripts harbor Short Interspersed DEgenerated Retroposons (SIDER1 and SIDER2 subfamilies) in their 3'UTR and that these truncated retroposon elements are major PTR players with a high potential of diversified regulatory functions. We have shown that SIDER2 elements promote co-translational mRNA decay through endonucleolytic cleavage and contribute to stage-specific gene expression. As confirmed by Illumina and Nanopore whole-genome sequencing, 1127 among the 1449 SIDER2 elements found in the *Leishmania infantum* genome were mapped within 3'UTRs. Next-generation sequencing and bioinformatic analysis of the *L. infantum* promastigote transcriptome indicated that ~70% of SIDER2-containing mRNAs were expressed at lower levels than the average expression in total transcriptome, hence supporting our previous

data that SIDER2 retroposons are generally associated with mRNA degradation. Some functional clustering was observed within defined expression subgroups of SIDER2-containing transcripts, which may indicate the presence of RNA regulons. Comparative analysis between *L. infantum* promastigote and axenic amastigote transcriptomes revealed ~37% of developmentally regulated SIDER2-containing transcripts. On the other hand, 20% of SIDER2 transcripts were differentially regulated in macrophage-derived amastigotes. Most of the observed changes in expression occurred in response to heat and acidic pH stress, known to trigger amastigote differentiation. However, differential regulation of a smaller number (7.4%) of SIDER2-containing transcripts was triggered by other intracellular signals. Experiments are currently under way to assess the cause(s) of developmental activation/deactivation of SIDER2-mediated mRNA decay. Our previous data suggest that stage-specific SIDER2 inactivation correlates with the absence of endonucleolytic cleavage. Changes in SIDER2 RNA structure or differential regulation/binding of ribonucleases and their auxiliary factors could prevent or favor mRNA cleavage and degradation. To characterize the SIDER2 RNA degradosome, we used a combination of MS2-MCP tethering assays, immunoprecipitation, proximity labeling and mass spectrometry studies. We have currently identified ~20 putative components of the SIDER2 RNA degradosome, including PIN-domain endoribonucleases, 5'-3' and 3'-5' exoribonucleases, Pumilio (PUF) proteins and other RNA-binding proteins of unknown function, and RNA helicases of the DEAD-box family. Globally, these studies will advance fundamental knowledge into yet unexplored central PTR mechanisms regulating mRNA abundance by the most preeminent cis-acting regulators in *Leishmania,* whose uniqueness could lead to novel therapeutic interventions.

S11-02: TRANSLATION REGULATION OF RIBOSOMAL PROTEIN mRNAs IN *Leishmania*: NOVEL ROLES FOR AN eIF4F-LIKE COMPLEX AND ASSOCIATED RNA-BINDING PROTEINS

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Leishmania and related protozoans are known for their lack of regulation of mRNA synthesis, generally the major stage for the control of gene expression in living cells. Regulation of mRNA translation is therefore even more critical for survival and adaptation to different extracellular conditions than most other eukaryotes or even prokaryotes. These pathogens can thus be considered excellent models for the study of events associated with the metabolism of mRNAs and their regulation. Most of these events are likely dependent on RBPs, a large number of which are found in these organisms, such as the cytoplasmic Poly-A Binding Protein (PABP). This is a major eukaryotic RBPs that binds to the 3' ends of nearly all mature mRNAs and is known to have with multiple roles associated with mRNA processing, transport, stability and translation. PABPs have been characterized mainly from multicellular organisms and yeasts, with a variable number of PABP homologues seen in different organisms, although the biological reasons for multiple PABPs are generally not well understood. A unique aspect concerning the initiation of the mRNA translation in *Leishmania*, also studied in *Trypanosoma* species, is the requirement for multiple eIF4F-like complexes. The eukaryotic eIF4F is formed by the union of two main subunits, eIF4E, the cap binding protein, and eIF4G, a large

multidomain protein involved in a number of interactions with different protein partners and which are required for efficient mRNA translation. Six eIF4E and five eIF4G homologues have been found conserved in *Leishmania* and related species, with different homologues joining specifically to form the distinct eIF4F complexes whose functional roles have only recently started to be defined. The evidence so far suggest that different complexes might be associated with different protein partners and distinct groups of mRNAs targets, possibly linked to specific regulatory events. Indeed, results from *Trypanosoma brucei* have already shown a specific association of one of these complexes (based on the EIF4E6/EIF4G5 subunits) with a specific subset of mRNAs, those encoding VSGs (Variant Surface Glycoproteins), while we have also seen an association of a second complex (EIF4E4/EIF4G3) with mRNAs encoding ribosomal proteins. Ribosomes are responsible for the major catalytic events associated with the mRNA translation and protein synthesis in all living organisms. These are small but abundant organelles which are strictly required for growth and survival. In eukaryotes, over eighty individual proteins are required for the formation of the two ribosomal subunits. Synthesis of these ribosomal proteins therefore is one of the greatest cellular demands in energy and nutrients and it is tightly regulated, generally through mechanisms controlling translation of their mRNAs. Diverse mechanisms have been shown to act regulation of translation of mRNAs encoding ribosomal proteins, from bacteria to mammals. In the latter, this regulation is mediated by a specific RNA binding protein (RBP), named LARP1, that binds to a motif consisting of a polypyrimidine tract (TOP) found at the 5' end of the targeted mRNAs. Three PABPs are found in *Leishmania*, more than in other unicellular organisms, with presumably distinct but not clearly defined roles. In order to better define their function, we sought to identify different mRNAs targets and specific RBPs with whom they interact. Using RNA-immunoprecipitation sequencing analysis we showed that the *Leishmania* PABP1 preferentially associates with mRNAs encoding ribosomal proteins, while PABP2 and PABP3 bind to an overlapping set of mRNAs distinct to those enriched in PABP1. Immunoprecipitation studies combined to mass-spectrometry analysis identified RBPs differentially associated with PABP1 or PABP2, including RBP23 and DRBD2, respectively, that were investigated further. Both RBP23 and DRBD2 bind directly to the three PABPs *in vitro*,

but reciprocal experiments confirmed preferential co-immunoprecipitation of PABP1, as well as the EIF4E4/EIF4G3 based translation initiation complex, with RBP23. Other RBP23 binding partners also imply a direct role in translation. DRBD2, in contrast, co-immunoprecipitated with PABP2, PABP3 and with RBPs unrelated to translation. Over 90% of the RBP23-bound mRNAs code for ribosomal proteins, mainly absent from the transcripts co-precipitated with DRBD2. These experiments suggest a novel and specific route for translation of the ribosomal protein mRNAs, mediated by RBP23, PABP1 and the associated EIF4E4/EIF4G3 complex. We proposed that RBP23 marks the ribosomal protein mRNAs, by binding to common motifs shared by these transcripts, as targets for translation mediated by PABP1 and the EIF4E4/EIF4G3 complex. Preferential binding of this eIF4F complex to the mRNAs encoding ribosomal proteins would direct them to a translation pathway distinct from most other cellular mRNAs, perhaps avoiding competition and in agreement with these mRNAs behaving distinctively than most other messages during translation. In mammals, LARP1 was found to act as a translational repressor that binds to PABP and consequently form a translation-inactive mRNA loop. Its phosphorylation releases it from targeted mRNAs and allows eIF4F assembly and translation. In *Leishmania*, we found that both PABP1 and EIF4E4 are simultaneously phosphorylated during exponential growth, coinciding with maximal protein synthesis and presumably activation of translation of their target mRNAs. Using site-directed mutagenesis, we mapped their phosphorylation sites and found those to be compatible with a phosphorylation by MAP kinases or cell-cycle regulated kinases. Indeed, orthologues in *T. brucei* for both proteins were found to be phosphorylated by the same cell-cycle regulated kinase, CRK1. The phosphorylated residues, for EIF4E4 at least, are in close proximity with motifs found to mediate its binding to PABP1, a novel and direct interaction not found in eIF4E or PABP homologues from other eukaryotes. It seems likely that the simultaneous phosphorylation of both proteins either enhances or inhibits their interaction, an event directly linked to the enhanced translation of their targeted mRNAs during the *Leishmania* exponential growth. Overall, our results define a specific association between one of the *Leishmania* PABPs (PABP1), a single RBP (RBP23) and mRNAs encoding ribosomal proteins, an important subset of cellular mRNAs. They also shed new light on the interactions required for

the translation of these mRNAs, highlighting the relevance of regulating their translation in different organisms and the convergence of regulatory mechanisms acting through their 5' and 3' ends and acting on the ribosomal protein mRNAs. The data also expands on the known PABP roles, with PABP1 functioning through mechanisms not seen elsewhere. The novel interactions identified, with further future characterization, might constitute possible targets for new and specific inhibitors of translation in *Leishmania* and closely related pathogens.

S11-03: INVESTIGATING *TRANS*-REGULATORS OF THE *Leishmania* LIFECYCLE

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Leishmaniasis cases are spreading globally, no vaccine is currently available and treatments are limited with toxic side effects and growing resistances. *Leishmania* are dixenic parasites that transition between extracellular stages in the sandfly to an intracellular stage that infects and destroys human immune cells. Fundamental parasite processes that evolutionarily diverge from human hosts can inform new intervention strategies. Lifecycle progression requires tightly-coordinated gene expression that preadapts *Leishmania* parasites for survival and virulence. Kinetoplastid gene regulation is predominantly reliant on post-transcriptional mechanisms. Accordingly, RNA binding proteins (RBPs) have an elevated importance for gene regulation in these organisms. Despite this, strikingly few *trans*-regulators have been characterised in *Leishmania* to date. Recently we isolated the mRBPome of the 3 main *Leishmania* lifecycle stages and found that RBPs selectively bind distinct transcriptomes in a stage-specific manner (Pablos, Ferreira et al., 2019). Moreover, a separate study revealed that arginine methylation of RBPs impacts target transcript association and stability in a method both bespoke and precise with epigenetic implications toward parasite virulence (Ferreira et al., 2020; Diniz et al., 2021). Building upon this work, 70 mRNA-bound RBPs were selected from a recent mRNA-bound proteome of the three main *L. mexicana* lifecycle stages. Through an optimised CRISPR strategy (Baker et al., 2021), a bar-coded *trans*-regulator knockout clone library was created and screened for essential roles in cellular differentiation, and macrophage/mouse infections. Of those screened, 40 mRBPs were incapable of creating viable knockout cell lines

and a further 18 contribute to lifecycle progression to human-infective stages and/or parasite infectivity. Examination of individual knockout lines for amastigote-specific mRBPs showed normal promastigote growth dynamics, whereas infection in peritoneal macrophages was inhibited, suggesting essential roles of RBPs for amastigote viability and virulence. Experiments are underway to identify the novel virulence pathways these RBPs regulate. Implications of these findings linking *Leishmania trans*-regulatory networks to eukaryotic post-transcriptional epigenetics will be discussed.

S11-04: DIFFERENTIALLY EXPRESSED NCRNAS IN *Leishmania braziliensis* ACT AS REGULATORY ELEMENTS AFFECTING GENE EXPRESSION AND DISTINCT CELLULAR PROCESSES

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Several classes of noncoding RNAs (ncRNAs) involved in a variety of regulatory networks have been described in physiological and pathological conditions in a diversity of organisms. Our laboratory is focused on understanding some of the layers at which regulation of gene expression occurs in *Leishmania*. Serendipitously, studying a group of short unannotated and polyadenylated transcripts from *Leishmania major*, we identified some transcripts arising from the 3'UTR of annotated protein coding genes. Subsequently, we conducted an in-depth study on the modulation of gene expression across the life cycle stages of *Leishmania braziliensis* covering coding and noncoding RNAs (ncRNAs). Analyses of differentially expressed (DE) genes revealed that most prominent differences were observed between the transcriptomes of insect and mammalian proliferative forms (6,576 genes). A computational pipeline and five ncRNA predictors allowed the identification of ~3,600 DE ncRNAs. These putative DE ncRNAs displayed a wide range of lengths, chromosomal distributions, and locations. To proceed with a functional analysis, a group of 18 DE long (lncRNAs) and short (sncRNAs) noncoding RNAs were selected, and the transcript length and processing features were confirmed/evaluated by a circularization assay for four of them. We learned that most of them have no Spliced Leader RNA at their 5'UTR ends and some of them do not carry a polyA+ posttranscriptional modification; the post-transcriptional processing mechanisms have not been identified so far. Seven ncRNAs, six lncRNAs and 1 sncRNA, were knocked out (KO) and five KO parasites presented altered features correlated with promastigote in vitro growth, metacyclogenesis, amastigote doubling time, nutritional or

oxidative stress resistance and macrophage in vitro infection profile. Proteins particularly binding to these ncRNAs were identified in *in vitro* pulldown assays using each ncRNA fused to S1m aptamer, *in vitro* transcription and streptavidin rescuing of ncRNA-bound proteins. In addition, the subcellular distribution the ncRNAs is under investigation. Our results indicate that some of the predicted DE ncRNAs disclosed in *L. braziliensis* transcriptome are involved in essential biological processes for the parasite to proceed successfully throughout its life cycle.

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S12. VL-HIV COINFECTION

S12-01: SECONDARY PROPHYLAXIS IN VL/HIV COINFECTION: WHO TO START AND WHEN TO STOP

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Coinfection with the human immunodeficiency virus (HIV) and *Leishmania* has been an emergent problem in the last three decades It occurs in 35 contries in the tropics, subtropics, and southern Europe, predominantly as visceral leishmaniasis (VL).

Leishmania/HIV coinfection impairs immune responses, increases treatment failure and relapse rates. The introduction of HAART resulted in the decrease of *Leishmania*/HIV coinfection cases; nevertheless, the number of relapses remains high and secondary prophylaxis (SP) is generally recommended. However, SP is not necessary in all patients, and presents a high risk of toxicity and an elevated cost. In general, it is preferable to maintain SP only in those patients who show predicting factors of relapse or treatment failure like the incapability of the patient to increase the CD4 cell count, having a history of previous relapses, and a CD4 cell count ≤100 cel/µl. In addition, there are no secure recommendations for the maintenance of SP. While some experts recommend maintaining it indefinitely, some others suggest to withdraw it in selected patients who have >200–350 cel/µl CD4 and have not presented any relapses in the last 6 months, with undetectable viral load for more than 3 months, and preferably with negative PCR for *Leishmania* in blood. However, the presence of other factors than CD4 cell levels might also affect the therapy response, and a

secure withdrawal of SP. In VL immunocompetent patients, the successful response to therapy broadly depends on the activation of the IFN-, which produces a Th1 subset of CD4 positive *Leishmania*-specific T cells, resulting in a complete cure without relapses. For that reason, cell-mediated immunity assays against *Leishmania* have been proposed to help in the decision of the secondary prophylaxis´ withdrawn. We performed the follow-up of 11 *Leishmania*/HIV patients living in Spain during 5-10 years. Blood samples were used to perform cell proliferation assay and whole blood assay (both using soluble antigen of *Leishmania*), IFAT serology, and quantitative PCR for *Leishmania*. The study showed that 7 out of 11 patients presented strong and specific cell-mediated immunity after treatment. All of these patients were left without prophylaxis for at least 24 months and presented no relapses (3 of them ≤200 CD4 cel/µl). The other 4 patients with negative cell-mediated tests, continued with secondary prophylaxis. During the follow-up, 3 of these 4 patients developed consistent cellular immunity against *Leishmania* and SP was withdrawn without no relapse. In consequence, a strong cell-mediated immunity against *Leishmania* allowed to safely withdrawn SP in 71% (6/7) of the *Leishmania*/HIV patients with ≤200 CD4 cel/µl. In conclusion, the performance of methods that assess cell-mediated immune responses may enable to withdraw the prophylaxis in *Leishmania*/HIV patients with ≤200 cel/µl CD4 cell counts. This could diminish the administration of drugs with many side effects, that are expensive, and which have proven to be insufficient to prevent relapses. In spite of the small number of patients of this observational study, our findings are consistent and promising enough to be investigated in future trials.

Keywords SECONDARY PROPHYLAXIS; HIV; *Leishmania infantum*; VL; CYTOKINES.

Financing CIBERINFECT; RICET (RD16CIII/0003/0002); AESI (PI18CIII/00029)

S12-02: PREDICTING VISCERAL LEISHMANIASIS IN HIV INFECTED PATIENTS IN ETHIOPIA: FIRST STEP TOWARDS A SCREEN AND TREAT STRATEGY

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HIV coinfection is one of the key challenges for control and management of visceral leishmaniasis (VL). VL-HIV coinfection rates are particularly high in NW-Ethiopia, reaching 20-40% of all VL cases. Once *Leishmania donovani* infection has evolved to the disease stage VL, prognosis at the individual level is dire, with many patients experiencing frequent relapses. Tackling *Leishmania* infection before disease onset would thus be a logical approach. We hypothesized that the period of asymptomatic *Leishmania* infection constitutes a window of opportunity for screening strategies, to capture those at high risk of developing VL. To build the evidence-base for such a strategy, we conducted a prospective cohort study including HIV-positive adults enrolled in HIV care in a VL endemic region in North-Ethiopia. Patients were monitored for *Leishmania* infection and VL development up to two years with clinical and laboratory evaluations every three to six months. Laboratory evaluations included rK39 RDT, rK39 ELISA, DAT, KAtex, *Leishmania* kDNA PCR, CD4 count and HIV viral load. Prevalent *Leishmania* infection was defined as positivity on any of the *Leishmania* markers at baseline, incident infection as positivity on any marker during follow-up in those with negative markers at baseline. Risk factors for VL were identified using Cox regression. The study was conducted between October 2017 and October 2021. A total of 571 individuals have been recruited and followed up to two years of which 34 (6%) have developed VL. Data cleaning is currently ongoing and statistical analysis is planned to start February 2022. Final results will be available at the time of WL7. We will present a prognostic tool to allow for individual prediction of VL risk at each point during clinical follow-up. After defining which HIV patients are at highest risk of developing VL, these patients can be targeted for prophylactic treatment in a follow-up clinical trial.

S12-03: IMMUNOLOGICAL DETERMINANTS OF RECURRENT VISCERAL LEISHMANIASIS IN HIV PATIENTS: BEYOND CD4 T CELL COUNT

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Despite apparent parasitological cure and successful virological suppression, more than half of HIV-coinfected visceral leishmaniasis (VL) patients in NW-Ethiopia exhibit a disease course characterized by frequent VL relapse. The causal pathway underlying this synergistic disease course remains underresearched and is often accompanied with an incremental treatment unresponsiveness. Due to the lack of well-defined predictive biomarkers or algorithms of VL relapse to guide clinical decision making, treatment optimization remains empirical. To date, only a few immunological determinants of recurrent VL relapse have been identified, such as a persistent lack of CD4+ T cell reconstitution. During this talk, first an overview of described immunological changes in VL-HIV patients will be presented and how it underlies a more chronic disease course. In addition, we will present recent compositional and functional single cell profiling data of the cellular immune landscape in 60 selected patients of the PreLeisH cohort in North-West Ethiopia. This unique cohort of >500 HIV patients was monitored over time to study the progression from asymptomatic infection towards active VL and includes a longitudinal immunological characterization of the disease course of >10 chronic patients experiencing recurrent VL relapse. With single cell resolution, including joined transcriptomic and TCR profiling, we observed marked functional differences in CD4+, CD8+ T cells and antigen presenting cells between relapsing or successfully curing VL-HIV patients already at time of active disease development, indicating early prognostic value. In addition, after successful treatment, higher immune activation and T cell expansion was demonstrated in the CD4+ and CD8+ T cell compartments of the cured group at both the transcriptional and clonotype level. In general, relapsing VL-HIV

patients show a persistent host immune dysregulation affecting mostly the antigen-presentation-recognition axis, while those VL-HIV patients achieving successful cure were able to launch a functional protective immune response. Lastly, we define the next steps and argue for a warranted roadmap to combined immunochemotherapy and better host-based tools for clinical decision making in VL-HIV patients.

S13. “ATYPICAL" CUTANEOUS LEISHMANIASIS

S13-01: ATYPICAL CLINICAL FORMS OF LEISHMANIASIS IN COCHABAMBA, BOLIVIA

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The Leishmaniasis in Bolivia represents a 70% of its territory corresponding to tropical and subtropical areas of the country. Cutaneous Leishmaniasis, the most common clinical presentation, is caused by *L braziliensis* in 85% of cases and the remaining 15% of infection is produced by: *L. amazonensis; L. lainsoni* and L. *guyanensis*. In variable percentages for each species. In Bolivia, there are no a clinical criterion established by the Ministry of Health for differentiating cutaneous leishmaniasis from other skin conditions. In this way, the *Centro Universitario de Medicina Tropical* (CUMETROP) carried out a first characterization of tropical skin ulcers in the region of Cochabamba, Bolivia. With the interest of discriminating between cutaneous leishmaniasis and other tropical skin conditions, was developed a clinical classification system for the forms of presentation of Bolivian cutaneous leishmaniasis. This classification initially covered 5 clinical forms defined as: "Classic" ulcerative form which consists of a shallow ulcer with defined edges and a circular shape that represents 63% of cases of cutaneous leishmaniasis in the region of Cochabamba, Bolivia. The remaining 37% is made up of atypical forms of presentation of cutaneous leishmaniasis that, according to the CUMETROP classification, are identified as: Infiltrative forms which appear as flat lesions with poorly defined borders, represent 7% of cases. The proliferative forms presented as lesions in high relief with a well-defined border, represent 6% of the cases. The nodular forms are nodules with the particularity of presenting a small continuity solution in

the centre of the nodule, they represent 16% of the cases. Warty forms characterized by an irregular surface in high relief with defined edges, which represent 5% of cases. Later, as a result of clinical observations in several patients, the ulcerative form with lymphangitis was added to this classification. Its characteristics are similar to the classic ulcerative ones accompanied by lymphangitis, which represents 2%; As a complementary characteristic, pain is presented in the path of lymphatic involvement. Both verrucous and ulcerative forms accompanied by lymphangitis require a second course of treatment with pentavalent antimonials. As another atypical form, but of mucosal leishmaniasis, very rare, there are lesions considered without a portal of entry, that is, development of mucosal leishmaniasis without previous evidence of cutaneous leishmaniasis with treatment or spontaneous healing. At present, the reason for the presence of these atypical forms of cutaneous and mucosal leishmaniasis is unknown, but a dysfunctional immune response is hypothesized, as well as the virulence of the leishmania species circulating in Bolivia.

S13-02: IMMUNOPATHOLOGICAL STUDIES OF NON-ULCERATED CUTANEOUS LEISHMANIASIS BY *Leishmania (L.) infantum chagasi*

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Non-ulcerated cutaneous leishmaniasis (NUCL) caused by *Leishmania (L.) infantum chagasi* has been described in Central America in countries like as Nicaragua, Honduras, El Salvador and Costa Rica. Patients affected by NUCL do not report a previous history of visceral leishmaniasis. Clinically, these cutaneous lesions are characterized by papules or nodules that do not ulcerate regardless of the evolution time of the infection. The skin lesions are small in size between 5 and 10 mm, occur in exposed areas of the body, and they may be surrounded by a hypopigmented halo. Histologically, the cutnaoeus lesions are characterized by a mononuclear inflammatory infiltrate in the dermis that varies in intensity, with a predominance of lymphocytes followed by macrophages forming epithelioid granulomas with scarce parasitism. *In situ* studies using double-label immunohistochemistry showed a predominance of CD8+ and CD4+ T lymphocytes positive for IFN-γ+. The involvement of others lymphocytes subpopulations in the skin lesion of NUCL, as regulatory T cells (FoxP3+) and Th17 cells (RorγT+) was discreet. Among to the antigen presenting cells (APC), a predominance of M1 (CD68+/NOS2+) in relation to M2 (CD163+/IL-10+) macrophages subtype was observed; as well as higher number of CD1a+/IL-12+ in relation to CD1a+/IL-10+ Langerharns cells and CD11c+/IL-12+ in relation to CD11c+/IL-10+ dermal dendritic cells. The results show that the cellular imune response in skin lesions caused of NUCL caused by *L. (L.) infantum chagasi* is predominantly inflammatory that may be responsible for the control of the evolution of lesion size and tissue parasitism. However, additional studies are needed to assess the role of host genetic and immunological background, as well as diferences in the parasite

subpopulations, responsable to determine this rare clinical presentation of cutaneous leishmaniasis caused by *Leishmania (L.) infantum chagasi*.

Keywords *L.* (*L.*) *infantum chagasi;* NON-ULCERATED CUTANEOUS LEISHMANIASIS; IMMUNOHISTOCHEMISTRY; IMMUNOPATHOLOGY; CELLULAR IMMUNE RESPONSE

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S13-03: CHRONIC RELAPSING CUTANEOUS LEISHMANIASIS (CRCL)

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The classic skin lesions of leishmaniasis in the New World are generally single or few ulcers, small in size (less than 5 cm in diameter), of short evolution (3 to 9 months) and that respond to antileishmanial drugs in most patients. Between 2011 and 2020, at the Jorochito Dermatological Hospital (JDH) in Santa Cruz, Bolivia, we treated 43 patients with extensive skin lesions that simultaneously compromised several body segments and who had already been treated with pentavalent antimonials (PA) without achieving cure. They came from the endemic regions where patients with localized cutaneous leishmaniasis (CL) usually come to us without being able to detect an unusual concentration in a specific region. 17 years was the longest evolution time and this patient had already been treated 3 times for CL with partial improvement and relapses. The patient with the shortest evolution had 2 years and had already received two full treatment courses with PA.

In Table 1 we show the demographic and clinical characteristics of these patients and compare them with localized LC patients treated on the same dates.

	Patients (n=43)	Controls (n=45)
Gender ratio	10 / 1	5 / 1
Age in years (range)	36 (22 – 73)	22 (4 – 63)
Illness duration in months (range)	54 (22 – 204)	5 (1 – 11)
Number of lesions (range)	11 (5 – 22)	1 (1 – 4)

Total area of lesions (range)	286 cm2 (62,5 – 1.845)	7,2 cm2 (2,5 – 39,6)
Type of lesions	Multiple warty plates with some ulcerations alternated with healthy and scarred zones in the same lesion	Unique ulcer (75%) and infiltrated plaques (17%)
Body areas simultaneously affected by lesions (range)	3 (2 – 6)	1 (1 – 2)
Comorbidities Diabetes II Overweight Hypertension HIV / Immunosuppression	 3 8 5 0	 1 2 4 0

There were no other remarkable abnormal laboratory results except blood glucose in diabetic subjects.

Table 2 shows the results of the specific tests for leishmaniasis of both, subjects and controls.

	Patients (43)		Controls (45)	
	Pos/total	%	Pos/total	%
Direct smear	29/43	67	32/45	71
IDR	23/32	72	29/41	71
Biopsy	12/18	67	9/15	60
Culture	11/14	78	9/13	69
Specie Id. *	7/7	100	5/8	75
LRV-1	2/3	66	1/4	25

* All isolates were identified as *L. (V.) braziliensis*.

Response to treatment

First treatment: IM or IV PA 20 mg/kg/d x 20 days was used in all patients. Correct complete scheme: 27 / 43 (62.8%); the errors made were pauses of 1 to 4 days due to pain at IM injection sites or a lower daily dose (12.4 mg/kg/d). Results: 30 (70%) presented initial improvement from 51 to 75% and 13 (30%) showed no change or worsened in relation to the initial evaluation. Between 2 and 5 months after finishing treatment, all of had reactivated lesions.

Second treatment: All received IM or IV PA 20 mg/kilo/day and 21 (49%) initially improved, but all returned to present active lesions with some positive parasitological examination between 4th and 8th month of follow-up.

Third treatment: 20 received IM or IV PA; in 10 same previous dose was used and only in 3 there was some improvement. In 3/6 who used PA with no upper limit of 3 amps (average 25 mg/kg/d) and in 2/4 who used 20 x 20 but for a period of 30 days, there was initial improvement and then relapse. 4/11 patients who received Amphotericin B deoxycholate at 0.8 mg/kg/d for 35 days and 3/5 who received miltefosine at 2.2 mg/kg/d for 28 days showed initial partial improvement.

Other systemic monotherapy: During the 4th or 5th course of treatment, any of the following was used: oral miltefosine (2.2 mg/kg/d for 28 days), IM pentamidine (4 mg/kg/d x 7 doses) or liposomal amphotericin B (2.3 mg/kg/d per 20 doses).

Other systemic treatments in combination: During the 4th, 5th, 6th or more treatment courses, any of the following combinations were used: oral miltefosine (2.2 mg/kg/d for 28 days) plus pentoxifylline (1,200 mg/d for 28 days). IV PA 20 mg/kg/d x 20 days plus pentoxifylline (1,200 mg/d for 30 days), IM pentamidine (4 mg/kg/d x 11 doses) plus oral miltefosine (2.2 mg/kg/d for 28 days) or liposomal amphotericin B (2.3 mg/kg/d for 20 doses) plus oral miltefosine (2.2 mg/kg/d for 28 days).

Other combinations: In 2 patients who had already undergone 6 courses of treatment and in 1 who had undergone 7 courses, two systemic medications and a local therapy (intralesional AP or cryotherapy) were combined.

These patients had a moderate good initial response to different antileishmanial drugs but will relapse in the following 4 to 15 months. This and the fact that the skin lesions are large warty plaques that are frequently ulcerated and that in the same lesion scar areas are found alternating with active lesions and with healthy skin, that mucosal involvement is the exception and not the rule, and that parasites are visible in most patients are what differentiates these patients from those suffering from disseminated cutaneous leishmaniasis.

Whether this clinical presentation is the result of changes in parasite genetics, parasite viral infection, patient immunological status or comorbidity, environmental changes, or a combination of all these factors, is something that has yet to be defined and should be studied.

S13-04: COMORBIDITIES IN CHRONIC CUTANEOUS LEISHMANIASIS IN LATIN AMERICAN

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The clinic presentation and response to therapy in cutaneous leishmaniasis (CL) due to *Leishmania (Viannia) braziliensis* is quite variable. Comorbidities may be associated to atypical lesions as flat, superficial ulcers with not well-defined borders, large and ugly ulcers, multiple nodules localized in one segment of the body, exuberant exophytic lesions or hypertrophic ulcers. Here we describe how diabetes melittus, pregnancy and obesity may modify the clinic presentation of CL, the abnormalities in immunologic response responsible for these modifications and how comorbidities increase the rate of therapeutic failure to meglumine antimoniate therapy. Pregnancy is not a morbidity but as the fetus has about half of the antigens from the father it is important that pregnant women develop mechanisms to avoid fetus rejection. Some pregnant women may present typical CL ulcers, but some present exophytic lesions. Compared to non-pregnant women who have an inflammatory reaction mediated mainly by CD4+T cells expressing IFN-γ and macrophages, pregnant women have a more intense inflammatory reaction and CD4+ T cells expressing IL-4 is the predominant cellular type in the lesion.

The diabetes mellitus impairs the immune response to bacteria and fungi mainly due to impair the ability of neutrophils to kill infectious agents. The leishmaniasis are protozoa caused diseases and the defense mechanisms is mediated by macrophages activated by $CD4^+$ Th1 cells. We compare the clinic presentation of CL in patients with and without diabetes and we found that about 40% of the patients with diabetes and CL presents atypical

lesions, mainly characterized by flat ulcers without defined borders, what is quite different from the classical well limited ulcers with raised borders observed in the majority of CL patients. While there was no difference in the production of IFN-γ between the 2 groups, patients with atypical ulcers produce higher levels of IL-1β, IL-6 and TNF than CL patients without diabetes. While there was no difference in the response to therapy comparing CL with diabetes versus those without diabetes, the cure rate in diabetic patients with atypical lesions was 33% while in diabetics with typical CL lesions was 81% (P<.05).

Obesity is on rise in the whole world and obesity has been observed in the poorer population like people that live in endemic areas of leishmaniasis. In the endemic area of Corte de Pedra the prevalence of obesity is 18% and of overweight is 21%. Obesity may present atypical lesions characterized by hypertrophic ulcers predominantly above the belt. We have not found differences in the production of cytokines in obese and lean CL patients, but the cellular infiltrate is more intense in obese and in these patients the adipose tissue is infiltrated by neutrophils, macrophages and lymphocytes and phagocytosis and destruction of adipocytes are observed. Moreover, adipose CL produce higher leptin levels and have more neutrophils in the tissue than lean CL patients. The cellular infiltrate in the tissue is greater in obese than in CL, have more neutrophils and killing of adipocytes are documented the adipose tissue. Moreover, while the cure with meglumine antimoniate in lean CL patients is observed in up to 73%, in obese only 19% are cured with one course of antimony therapy. Different inflammatory pathways participate in the ulcer development of CL and obese, diabetics and pregnant women present different clinical presentation and poor response to therapy with meglumine antimoniate therapy.

S14. EPIDEMIOLOGY OF LEISHMANIASIS IN AMERICA

S14-01: SURVEILLANCE STRATEGIES FOR LEISHMANIASIS IN THE SOUTHERN CONE OF AMERICA BASED ON VECTOR ECOEPIDEMIOLOGY

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Based on field and modeling studies conducted by researchers of the Leishmaniasis Research Network of Argentina-REDILA-, an integrated vector surveillance sustainable proposals were developed for cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) in the Southern Cone of America involving different spatial scales. For this purpose, we take into consideration the theoretical framework of eco-epidemiology, information-based decision-making tools and integrated vector management, intersectoral coordination according to the One Health approach, and the need for empowerment of affected populations. For CL, based on the exposure associated with the edge effect, a multiscale environmental change surveillance network strategy stratified by risk was proposed, with at least four levels of actors, with different degree of responsibility: (A) international level, such as development project funding agencies, which should include CL risk assessment and monitoring among the requirements and impact assessment; (B) national level or first subnational jurisdictions, in addition to the health sector, areas involved in environmental change or population interaction with edges, such as forest regulatory agencies, meteorological monitoring and remote sensing agencies for natural disasters, armed forces involved in forest activities, environmental non-

governmental organizations; (C) in the municipal level, such as those responsible for urban development, civil defense, labor unions, and primary health care agents; and (D) actors living in the focus, individual volunteers, media, and social networks. For urban LV, we propose three decision levels for intervention, based on the distribution and persistence of intra-urban risk hotspots: (A) Determination of areas most likely to sustain transmission at the "city" scale using secondary sources, such as remote sensing for environmental indicators, census data for demographic indicators, and human or veterinary case surveillance system data, if available, (B) Identification of the most likely critical areas among the possible areas identified and visualized as patches or islands on a map or satellite image, these critical areas or hot spots are defined by local actors according to their knowledge of the socio-environmental geography of the city, which in turn can delimit the macro-habitat of risk contributing to reduce the dimensions of the patches from the previous scale; (C) the most likely critical sites within the most likely critical areas, the peri-domicile and domicile where to place vector traps for surveillance or for intervention, will be identified using primary data derived from the field survey and primary health care information. Both strategies, for VL and CL, are undergoing field validation and cost-effectiveness evaluation.

S14-02: THE KEY TO UNDERSTANDING LEISHMANIASIS EPIDEMIOLOGY – THE ENZOOTIC CYCLE

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As more information on *Leishmania* infections in sand flies and vertebrates becomes available different enzootic patterns are perceivable. These can broadly be classified into two evolutionary paths as being specialists and generalists. This is a subject that has been extensively discussed in evolutionary biology for plants, insects, and vertebrates. Specialist parasites are species that infect a very limited range of hosts while generalists infect a wider range. There is evolutionary evidence that some generalist multicellular parasites originated from specialists. However, for dixenous parasites such as the Leishmaniinae a species may be a specialist or generalist in both hosts, a specialist for its vertebrate host, a generalist in vector(s) or vice versa. This concept plays a role in the epidemiology within changing and stable environments. For a vector transmitted parasite which host exerts the principal selective force and is thus responsible for it being specialist or generalist? However, is it the vector or the reservoir that is responsible for these patterns? There are arguments that a specialist is the result of evolution in a homogenous environment while some generalists results from evolving in a heterogenous environment. As leishmaniasis in man is a zoonotic disease it suggests these *Leishmania* are generalists in relation to their mammalian host. Suggesting that the intracellular environment is more homogenous than the vector's intestine to be able to be transmitted successfully. Based on this examples will be given of specialist and generalist parasite vector relationships and how this reflects in the epidemiology of different Leishmaniinae species and its relationship to their genetic diversity.

S14-03: ENVIRONMENTALLY FRIENDLY STRATEGY FOR THE CONTROL OF NATURAL BREEDING SITES OF *Lutzomyia* sp IN AN ENDEMIC FOCUS OF THE COLOMBIAN CARIBBEAN REGION

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Leishmaniasis are a group of diseases caused by protozoan parasites which are transmitted by the bite of infected female phlebotomine sandflies. These diseases are endemic in tropical and subtropical regions, distributed in almost 100 countries and three territories. In the Americas, the transmission dynamics of leishmaniasis have been associated with sylvatic and rural environments. However, in recent years the disease has occupied new urban and peri-urban transmission scenarios due to the integration of different social, economic and ecological factors. Currently, vector control measures to reduce the incidence of leishmaniasis are directed at the adult form of phlebotomine sandflies through the systematic application of insecticides, use of repellents and impregnation of nets, all with controversial results due to coverage, costs, ecological impact and community acceptance. Intervention in natural breeding sites and modifying the environmental conditions of the peridomicile in order to prevent breeding and/or feeding is considered a promising control strategy. As a public health problem, leishmaniasis requires the development of effective integrated vector management strategies for sand fly control. However, these strategies should be economically and environmentally sustainable and can be adapted to the knowledge gained about the behavior

of the vector and the biology of its immature forms of phlebotomine sandflies. This descriptive study, based on previous findings, proposes an environmentally friendly strategy for the control of immature forms of the vector, based on liming of phlebotomine sandfly breeding sites in an endemic focus in the Colombian Caribbean region. The municipality of Ovejas is located in the department of Sucre at 256 masl. According to Holdridge's classification, the vegetation corresponds to tropical dry forest and is located in the sub-region of the Serranía de San Jacinto. The area shows high anthropogenic activity, with grazing areas, small forest relicts and dominance of shrub and herbaceous species. Precipitation of approximately 1100 mm is recorded in April and October. Previous studies in this municipality focused on the search for immature forms of phlebotomine sandflies through the collection of soil samples from peridomiciliary vegetation. A total of 505 immatures were recovered, of which approximately 50% were collected from the base of the tree species *Cordia alba*. Liming consisted of the application in equal parts of 500 grams of Calcium Hydroxide with Magnesium (Ca (OH)2 Mg (OH)2 diluted in two liters of water on the base of the trees and the surface of the tree trunk. To contrast the effect of liming, a control zone and a treated zone were established. Changes in soil physicochemical composition and microarthropod composition associated with lime application were recorded. The concentration of the following soil elements A, L, Ar, P, MO, Ca, Mg, K, CICE, and pH were measured. In the municipality of Ovejas, 29 trees were selected in the control zone and 28 in the treated zone. 1000 g of soil from the base of the trees were collected, of which 500 g of soil were deposited in hatching pots for larvae and observed for 60 days. Simultaneously, 200 g were taken to establish the composition of microarthropods and 300 g for physicochemical analysis of the soil. To explore the possible statistical association between the number of phlebotomine larvae or sandflies adults and the forest fragment, a poisson mixed linear model was fitted for longitudinal data, using the number of larvae or sandflies adults as response variable and forest fragment as an explanatory variable. This variable has two levels: Fragment 1 tropical dry forest or control zone (F1bs-T) and Fragment 2 tropical dry forest or treated zone (F2bs-T). Statistical significance was considered important if the p-value is less than 0.05 and edge-value if such p-value is greater than 0.05

and less than 0.1. The model was also run to explore the possible association between the number of larvae and some soil variables that were measured during the study period (A, L, Ar, pH, MO, Ca, Mg, K, CICE, and P). A total of 54 larvae emerged from the rearing sites with 37 and 17 phlebotomine sandfly larvae in the control and treated zones, respectively. These larvae were identified as belonging to the species *Lutzomyia* (*Lu.*) *evansi, Lu. cayennensis, Lu. rangeliana, Lu. atroclavata and Lu. Micropiga*. The statistical analysis of the effect of lime comparing the number of larvae recovered from the control area and intervened in the period Dec - Oct 2016 including all tree species did not show significant statistical association (p-value =0.2713). In contrast when the number of larvae recovered during the application of lime was analyzed only for *Cordia alba* an important significant association was found (p-value = 0.020). There was also no statistically significant association between the number of larvae recovered and the month of application (p-value = 0.9344). However, due to the positive value of the estimate for forest fragment 1, there was a tendency to recover more larvae in the control area in relation to the area under intervention. During the period of application of lime, 116 resting adult sandflies were captured in the control area and 39 in the intervention area. In the analysis between the number of adults at rest recovered in the intervened area compared with the control, a limit statistical association was found (value of p = 0.0896). However, due to the positive value of the estimate for forest fragment 1, there was a tendency to recover more adults at rest in the control area in relation to the area under intervention. The composition of microarthropods were grouped into 5 Subphylum, 7 classes, 22 orders and 72 families. The order Hymenoptera represented 32% of all catches, 31.4% of which belonged to the family Formicidae. The soil texture of the study area was classified as a sandy loam soil. Liming produced an increase in pH, calcium, Potassium and a decrease of phosphorus in treated zone. Finally, the model was run to explore the possible association between the number of larvae and some soil variables (A, L, Ar, pH, MO, Ca, Mg, K, CICE, and P) no significant statistical relationship was observed between them and the number of larvae recovered in both forest fragments (F1bs-T and F2bs-T).However, when only the breeding sites associated with *Cordia alba* were considered, an edge association was observed between the number of phlebotomine larvae and organic matter (p-value = 0.0649). The

findings of the liming-based study show 1) A change in the microenvironmental conditions of phlebotomine sandflies breeding sites. 2) A reduction in the immature stages of the phlebotomine sandflies 3) The bleaching of the trees alters the resting behavior and 4) Liming does not affect the composition of microarthropods.

S14-05: EVIDENCE AND GAPS IN KNOWLEDGE FOR THE CONTROL OF VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) is rapidly spreading worldwide. It was estimated that around 300 thousand cases occur each year globally, with a case-fatality rate of approximately 10%. Despite this, many public health interventions are taken without a sound scientific basis. Like many other neglected diseases, VL has minimal investments in research, which should be directed to questions that have not yet been sufficiently answered to achieve the WHO 2021-30 Neglected Tropical Diseases road map goal to reduce mortality caused by the disease to less than 1%. The evidence map systematically organizes the knowledge available on the subject in the health context, making it accessible. It is instrumental in identifying evidence gaps, helping researchers to find relevant topics for future studies, and facilitating health policy planning are the advantages of using this tool. Evidence maps exhibit the visual representation of knowledge through products with user-friendly formats that contribute to health professionals, researchers, and policymakers. In this sense, the present study aims to develop an evidence map about VL and systematize evidence available in the literature. Thus the results of the evidence map for the prevention and control of VL are described. Systematic reviews (SRs) on VL were searched on MEDLINE/PubMed and the Virtual Health Library. After selection, each SR included was evaluated, characterized, and categorized by type of intervention and by results, according to the methodology offered by

BIREME/PAHO/WHO. Methodological quality was assessed using the AMSTAR2 tool to determine confidence in the evidence obtained. Data processing was performed using Tableau Software®. The evidence map consists of an interactive matrix of interventions and outcomes in bubble plot format. Interventions and outcomes are found in the lines and columns of the graph, respectively. The bubble size indicates the number of SRs that relate a given intervention to its respective outcome. The bubble color represents the confidence level of SRs. More information about the study(s) is obtained by pressing the mouse button over each bubble. The platform also allows filtering of the results. Graphs for intervention groups and related outcomes, considering only effects and the number of SRs, were also created. The searches resulted in 218 articles, of which 152 did not refer to VL or were not SRs. Of the remaining 66 articles, 33 were excluded because they did not refer to diagnosis, treatment, prognosis, or prevention and control in VL. Then, only 33 SRs were included and characterized. Concerning the level of confidence, one review was classified as moderate, four reviews were classified as low, and 28 reviews had a critically low confidence level. The critical and non-critical questions that represent weaknesses for SRs about VL will be discussed.

The evidence map is available on an interactive online platform through the electronic address: https://public.tableau.com/app/profile/bireme/viz/leishmaniose-visceral-en/evidence-map.

The interventions evaluated in the prevention and control group include dog culling, insecticide-impregnated dog collars, insecticide thermal fogging, insecticide spraying, permethrin spot-on formulation, and insecticidal spraying on houses (walls), insecticide-treated bednets, bednets, vaccine, and educational material. The SRs that evaluated the dog culling had conflicting results, especially regarding reducing the incidence of VL in humans and seroconversion in dogs and humans. The included studies show a significant reduction in the seroconversion or incidence of VL in areas where dog culling was carried out. Meanwhile, other investigations did not significantly differ between the intervention and control groups analyzed. Similarly, two SRs diverge on the effect of dog culling on human

seroconversion. At the same time, one of them had the results of a study in which reservoir control reduced seroconversion in humans. Another review concluded that this intervention does not provide protection. Three SRs analyzed insecticide-impregnated dog collars. One of the reviews indicated the absence of a significant difference between the incidence rates obtained in the intervention studies. The remaining reviews, including a meta-analysis, highlight the statistically significant reduction in VL incidence in dogs stimulated by collars with deltamethrin. On the other hand, this intervention presented conflicting results regarding seroconversion in humans. It was observed that insecticide thermal fogging and insecticides on walls of houses could reduce the density of sandflies and cause the death of sandflies, respectively. Still, the resulting impact depends on the characteristics of the houses where the application was made. Insecticidal spraying on houses promoted a reduction in the density of sandflies. However, it had no significant effect on seroconversion in humans. The use of insecticide-treated bednets reduced the density and landing of sandflies in humans besides causing the death of vectors. However, they do not significantly reduce seroconversion and the incidence of human VL. It was impossible to conclude about the use of bednets due to divergent results on the existence of a protective effect of this intervention. Permethrin spot-on formulation and the vaccine with fucose-mannose ligand antigen (FML) proved helpful in decreasing the seroconversion and the incidence of VL in dogs.

Health education actions using educational material improved the knowledge of health professionals and school-age children about VL. Dog culling associated with insecticide spraying on houses did not significantly reduce human seroconversion compared with control groups in two of the SRs included. One SR showed inconclusive results due to the individual study. There was protection, but methodological weaknesses include the absence of a control group and losses to follow-up. The inconclusive effect for reducing the incidence of human VL also occurred due to methodological weaknesses. Additionally, dog culling, treatment of human cases, and insecticide spraying on houses did not significantly reduce VL incidence in dogs and humans. The methodology used in constructing the evidence map allowed the identification and characterization of effective interventions for

VL. The majority of the included SRs had a critically low level of methodological quality, which reveals that the reviews produced on the subject may have disadvantaged precision and scope. The main critical weaknesses were the absence of a list of the excluded studies and the use of a proper technique to assess the risk of bias. Methodological flaws were found, especially in individual studies about prevention and control, where there is not enough evidence about necessary measures implemented around the world. Some topics for the knowledge of VL remain unclear like (a) The effects of prevention and control measures concerning the incidence of the disease in humans, (b) the applicability of biological vector control, (c) advances in research for the development of vaccines for dogs and humans, (d) the impacts of VL screening for blood transfusion and organ transplantation, (e) the effects of using nutritional supplements in the treatment, (f) the best diagnostic and treatment tools for other groups of immunosuppressed patients, except co-infected with HIV, (g) the cost-effectiveness analysis for interventions. These are the main gaps observed, which deserve attention from researchers. The main strengths in the elaboration of this evidence map are the compilation of information from SRs on VL and the assessment of their methodological quality. However, analysis based solely on SRs constitutes a weakness of the study since some of the identified gaps may have been elucidated by individual studies not yet integrated into this type of publication. The evidence map consisted of a matrix of interventions and outcomes involving prevention and control, diagnosis, treatment, prognosis, and combined interventions on VL. As for the methodological quality, it was observed that most SRs have a critically low level of confidence. These publications present promising results for diagnostic and treatment techniques but with critical methodological weaknesses, especially regarding preventive interventions. Finally, the evidence map for VL shows there is insufficient evidence to support most prevention and control interventions. It is also evident that systematic reviews in VL are scarce and were based, in many cases, on small or methodologically weak studies. The quality of evidence was low or very low in most systematic reviews.

S15. ANIMAL MODELS FOR VISCERAL LEISHMANIASIS: SUITABILITY AND APPLICATIONS

S15-01: THE BEAGLE DOG MODEL IN NEOLEISH® PRE-CLINICAL VACCINE DEVELOPMENT

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Zoonotic visceral leishmaniasis continues being a major veterinary and public health problem. Vaccines are required for *Leishmania infantum* infection control because the parasite confines itself to the bone marrow leading to relapses and asymptomatic carriers, including treated dogs. Limited safety and efficacy of current treatments urge for the search for vaccines. Pre-clinical vaccine development against zoonotic visceral leishmaniasis requires experimentation with the beagle dog model because this breed is genetically less heterogeneous. However, appropriate experimental design, lodging, handling, and other technical and ethical aspects are resource-demanding. The pre-clinical efficacy trials of the non-replicative antibiotic resistance-free Neoleish® DNA vaccine followed the UE and Spain animal experimentation ethical regulations, including approval by

the University of Zaragoza Ethics Committee. Dogs were lodged in groups of 5 in 18 m^2 kennels and fed *ad libitum*, samplings were performed under 20 mg/Kg medetomidine hydrochloride anesthesia reversed with 100 mg/Kg atipamezole hydrochloride, and appropriate environmental enrichment was implemented including outside facilities for daily exercise, and euthanasia was performed with 50 mg/Kg pentobarbital sodium. Three pre-clinical tests were performed including a total of 108 dogs. The first test resulted in the pselection of the definitive immunization guideline, which consisted of homologous plasmid-plasmid prime-boost immunization by the intranasal route in a 15-day interval. The infectious challenge consisted of 10^8 promastigotes by the intravenous route 15 days post-booster dose. Circulating specific IgG total, IgG1, and IgG2a were assessed by ELISA. Lymphoblastic transformation tests (LTT) were performed with PBMC. The Th1/Th2 cytokine profile was evaluated in LTT supernatants by ELISA and in popliteal lymph node tissue by qRT-PCR. Differential gene expression between unprotected and protected dogs was assessed with GeneChip™ Canine Genome 2.0 Array. Pre- and post-challenge samples were considered in these immune response analyses. The clinical profile was based on a cumulative clinical score including (temperature, body weight, alopecia, skin ulcerations, hyperkeratosis, conjunctivitis, mucosal anemia, onychogryphosis, epistaxis, muscular atrophy, lymphadenomegaly).

The parasite burden in bone marrow was quantified by qPCR in all time points throughout the experiment, and in target organs only at the endpoint. A significant increase of the Th1 response was observed in most vaccinated animals in the pre-challenge period. The parasite burden in bone marrow decreased at least 100-fold in 60% of vaccinated dogs, of which 50% were negative. The clinical score was significantly lower in vaccinated dogs. 300 days post-infection, IFN-© levels were significantly higher and IL-10 significantly lower in LTT supernatants, as well as the corresponding transcripts in poplyteal lymph node tissue (Mann Whitney U test, $\alpha = 0.05$). Up-regulated canine genes associated to a protective immune response were detected in most vaccinated dogs subject to *L. infantum* challenge (cathepsin G, CTSG; chemokine (C-C motif) ligand 5, CCL5; chemokine (C-C motif) receptor 5, CCR5; and midkine-like genes). Neoleish® estimated efficacy is 60% in terms of parasite burden reduction. This vaccine has

achieved high safety standards and the efficacy results have been confirmed in a successful double-blind clinical field trial.

Keywords CANINE LEISHMANIASIS; BEAGLE DOG MODEL; LACK; DNA VACCINE; EFFICACY

Financing CZ Vaccines (CZ Veterinaria S.A., Zendal Group); RETOS-COLABORACION (MINECO)

S15-02: RODENT MODELS OF SAND FLY-INITIATED VISCERAL LEISHMANIASIS EXPOSE IMPORTANT FACETS TO THE IMMUNE RESPONSE THAT GOVERN *Leishmania* DISSEMINATION AND TRANSMISSIBILITY

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Sand flies inoculate a small number of *Leishmania* parasites into host skin. In visceral leishmaniasis (VL), these parasites escape the initial adverse innate immune response of the host and disseminate to lymph nodes and visceral organs causing a deadly infection if not promptly treated. Vector-transmitted models of VL reveal sand fly bite-specific events that are vital for parasite survival and dissemination. Our research and that of others elucidated the contribution of vector-derived factors injected alongside parasites, including saliva, the promastigote secretory gel, exosomes and gut microbiota, to survival and augmentation of the severity of leishmaniasis[1]. At the bite site, an acute inflammatory response characterized by high levels of IL-1® drives a sustained neutrophilic response that governs parasite dissemination[2], and it is exacerbated in malnourished animals reinforcing its significance in early parasite establishment[3]. This inflammatory burst is indirectly controlled by prolonged bleeding imposed by hematophagous insects, another facet unique to *Leishmania* development after transmission by infected sand flies. We found that tissue resident macrophages and infiltrating monocytes scavenge extravascular erythrocytes that have leaked from vessels as a result of lacerations to capillaries by sand flies during blood feeding . This leads to induction of heme-oxygenase 1 (HO-1) that catabolizes heme releasing the anti-inflammatory by-product carbon monoxide (CO). CO acts globally at the bite site suppressing inflammation and promoting disease tolerance[4]. Yet another facet specific to vector-transmitted models of VL that should not be overlooked is our finding that a

proportion of parasites inoculated at the bite site are retained in the skin forming depots that ensure their transmissibility to uninfected sand flies[5,6]. Collectively, these studies reinforce the importance of vector sand flies in governing VL development, and demonstrate that their influence extends well beyond the production and transmission of infectious parasites.

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S15-03: ANIMAL MODELS TO STUDY LIVE-ATTENUATED *Leishmania* AS VACCINE CANDIDATES TO TACKLE VISCERAL LEISHMANIASIS

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Since there is no safe and effective vaccine to control leishmaniases in humans, it is urgent the need for new alternative prophylactic strategies to tackle these diseases. Explore protective immune response against visceral leishmaniasis (VL) is essential to the development of vaccine candidates for controling this fatal form of leishmaniasis. In this talk we will focus on the main advantages and limitations of pre-clinical VL experimental models using mice, hamsters and dogs to revisit live-attenuated *Leishmania* parasite as first-generation vaccine candidates. *L. infantum*-infected mice are widely used to explore the host immunological profile upon vaccination followed by infection challenge. This is possible thanks to commercially available immunological reagents to dissect Th1/Th2 profile markers in mice. The model is a good predictor for protective immune response, since it involves self-curing (oligosymptomatic or subclinical infection) type driven by IFN-g production. Additionally, VL physiopathology includes liver granuloma formation leading to the resolution of hepatic infection which could persist in the spleen. By contrast, despite the absence of an immunological panel, hamsters could mimic canine and human VL based on immune, clinicopathological and parasitological features. Immune markers can be

indirectly assessed by measuring transcript level using RT-qPCR. High humoral response do not ensure infection control in hamsters and a high IL-10 background could represent a trade-off as anti-inflammatory component. Hamsters vaccinated with live-attenuated dermotropic centrin-deficient *L. major* parasites produced protective response upon *L. donovani* challenge. It represents a good hope for antileishmanial heterologous vaccine aimed to pan-protection driven by common immunogenicity pathways. In this regard, we will share some results on the use of *L. mexicana* kharon1 knock-out mutants (Likh1$^{-/-}$) as live-attenuated parasites to protect against *L. infantum* challenge. A similar strategy is also being performed to verify the vaccine potential of a live-attenuated lysine deacetylase deficient *L. mexicana*. We will also explore the homologous use of *Likh1*$^{-/-}$ as vaccine strain against VL in mice. Like hamsters, dogs also mimic the human disease but its use as VL models could be prevented in limited resources scenarios. *L. infantum*-infected dogs can present high parasitemia being susceptible to the infection. Naturally-infected dogs can be used to evaluate the immunotherapeutic potential of a given antileishmanial vaccine. In this context, a good vaccine must ensure disease control leading to absence of symptoms and block parasite transmission, even without parasite clearance. Since *L. infantum*-infected dogs present high parasite load in the skin, it can be affordable to meet these model requirements. In summary, mice, hamster and dogs are complementary VL experimental models in which preclinical vaccine research should advance based on the achievements from each model and the level of complexity through the pipeline towards the market.

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S15-04: INCREASED LEVELS OF CORTISOL ARE ASSOCIATED WITH THE SEVERITY OF EXPERIMENTAL VISCERAL LEISHMANIASIS CAUSED BY *Leishmania (L.) infantum*

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Several infectious diseases are associated with hypothalamic-pituitary-adrenal (HPA) axis disorders by elevating circulating glucocorticoids (GCs), which are known to have an immunosuppressive potential. We first did a study in golden hamsters, a suitable model human visceral leishmaniasis (VL), to investigate the relationship of *Leishmania (L.) infantum* infection on cortisol production and VL severity. Increased parasite burden was associated with higher arginase expression and lower iNOS induction. Cortisol levels were elevated in infected animals in all-time points evaluated up to 180dpi. Except for monocytes, all other leucocytes showed a strong negative correlation with cortisol, while transaminases were positively correlated. Immunological markers as interleukin (IL)-6, IL-1β, IL-10, and transforming growth-factor-β (TGF-β) were positively correlated to cortisol production, while interferon-χ (IFN−γ) presented a negative correlation. A network analysis showed cortisol as an important knot linking clinical status and immunological parameters. Then, we decided to investigate the putative influence of *L. infantum* antigens on the mechanisms driving

glucocorticoid production by adrenals. *L. infantum*-infected Balb/c mice showed increased levels of plasma corticosterone at 5 dpi and a peak of hormone production at 30 dpi, in parallel with the rise of parasite burden in the spleen. Unexpectedly, in long term evaluation as 120 dpi there was a decrease of corticosterone levels, despite the high parasitic load in the spleen. The injection of ACTH induced an increase of corticosterone levels in both uninfected mice and infected mice. Despite presenting reduced MC2R receptor, adrenals from *L. infantum*-infected mice had a higher capacity to increase of corticosterone after ACTH stimulus (2.8-fold) than non-infected ones (1.9-fold). It demonstrates that adrenal function is preserved in infected mice during all time. Finally, also in mice model the cortisol levels were positively correlated with IL-6, a hallmark of severity in VL besides a potent GC inducer. In summary, our results showed that experimental VL evolve with hypercorticoidism since the early time after infection, probably not only as a direct effect of leishmanial antigens on adrenal glands, but also as a consequence of the immunopathological events related to the *L. infantum* infection. Whether the glucocorticoids influence or not the VL-associated immunesupression should be elucidated.

Keywords *Leishmania infantum*; CORTISOL; HAMSTER MODEL; VISCERAL LEISHMANIASIS; iNOS/ARGINASE; CYTOKINES; CLINICAL OUTCOME

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S16. DRUG RESISTANCE AND TREATMENT FAILURE IN LEISHMANIASIS: A 21ST CENTURY CHALLENGE

S16-01: PARASITE DRUG SUSCEPTIBILITY AND RESPONSE TO TREATMENT IN CUTANEOUS LEISHMANIASIS

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Despite the capacity of drugs used to treat leishmaniasis to kill the target parasite, the relationship between drug susceptibility of *Leishmania* and therapeutic response is uncertain for all forms of human leishmaniasis. The multifactorial basis of the outcome of treatment and the contribution of known and unknown risk factors of treatment failure confound attempts to correlate drug susceptibility with clinical response. This knowledge gap impedes the consideration of resistance in treatment policy or decisions and the optimal use of anti-leishmanial drugs to reduce morbidity attributable to infection, and to chemotherapy. Parasite survival and persistence of infection following treatment and resolution of lesions is common, having been demonstrated in healthy tissues at diverse and distant body sites as well as lesion scars in a high proportion of patients. Hence, clinical response to treatment is not contingent upon parasite elimination. Evaluation of drug susceptibility of strains isolated pre-treatment and at reactivation following therapeutic cure from the same patient, revealed acquired resistance to pentavalent antimonial drug as well as evidence of primary resistance, confirming the participation drug resistance in reactivation in 40% of the cases investigated. Nevertheless, the other cases of reactivation involved drug-sensitive infections, underscoring the multifactorial basis of therapeutic response in these as well as other microbial pathogens, and the

necessary, but not sufficient, participation of persistent infection. *Leishmania (V.) panamensis* populations circulating within the Pacific Coast Region of Colombia predominantly pertain to two discrete zymodemes. Analysis of drug susceptibility of clinical strains and correlation with previously determined zymodeme classification revealed that parasites belonging to zymodeme 2.2 and 2.3 respectively, were susceptible and intrinsically resistant to pentavalent antimony. Subsequent evaluation of the clinical outcome of antimonial treatment of CL caused by parasites pertaining to these zymodemes have shown that treatment failure occurred in a higher proportion of patients infected with L. (V) panamensis zymodeme 2.3 strains (21/35, 60%) than zymodeme 2.2 strains (13/34, 38%). We further found that parasites belonging to these zymodemes differentially modulated the inflammatory responses elicited in primary human macrophages and neutrophils, and differ in pathogenicity in BALB/c mice. Together these findings suggest that the distinct host responses to these parasites participate in their intrinsic susceptibility phenotype and clinical response to antimonial drug. To probe the relationship between antimony susceptibility and host cell response in therapeutic outcome, we concomitantly evaluated the susceptibility of clinical strains in primary human macrophages and PBMCs compared with the U937 histiocytic cell line conventionally used in determining phenotypic susceptibility to anti-leishmanial drugs. Parasite survival was evaluated in parallel by microscopy and quantitative PCR of *Leishmania* 7SLRNA, obtaining a high correlation of results obtained by the two methods. Although the disparate susceptibility of zymodemes 2.2 and 2.3 to pentavalent antimony was evident in all host cell models, discrimination of antimony susceptibility phenotype in relation with clinical outcome was most sensitive in the U937 human cell line ($p \leq 0.001$). Drug tolerance or resistance to antimony was found to be significantly higher among strains from patients with treatment failure compared with patients that cured following treatment, supporting the clinical and epidemiological relevance of in vitro susceptibility. Parasite survival during drug exposure in the ex vivo and in vitro models likely reflects the integrity of the inflammatory response of primary macrophages and PBMCs compared with the U937 cell line. Ongoing transcriptomic analyses seek to decipher the host cell contribution to susceptibility to antimonial drugs.

S16-02: CELLULAR AND PROTEOMIC CHARACTERISTICS OF PENTAVALENT ANTIMONY REFRACTORINESS IN *Leishmania guyanensis*

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Cutaneous (CL) and Mucosal (ML) leishmaniasis in the Americas are mainly related to infections with *Leishmania* species of the *Viannia* subgenus. *Leishmania guyanensis* is the species predominantly associated to CL in north-central Brazilian Amazon Region and in Guyana, Suriname and French Guyana. In these regions, *L. guyanensis* is responsible for different clinical

forms of CL including localized cutaneous leishmaniasis, lymphangitis, disseminated cutaneous forms, and also mucocutaneous cases. The first line treatment for CL and ML in Brazil is pentavalent antimonial - Sb^V (Meglumine antimoniate -Glucantime); however, in infections with *L. guyanensis* the treatment with this medication is associated with low cure rates (between 53% and 70%), imposing a challenge for the public health systems in the region. Due to the therapeutic failure observed with Sb^V, the current treatment choice involves administration of pentamidine isethionate despite the few evidence about its use. In the last decades, proteomics has contributed for understanding the molecular basis of the therapeutic failure observed in the treatment with Sb^V; however, most of the studies were made for Old World *Leishmania* species and little is known about the proteomics of antimony resistance in distantly related and important parasite species circulating in American Countries, such as *L. guyanensis*. Using cellular and molecular approaches, our group has studied *L. guyanensis* strains isolated from cured or refractory patients after treatment following recommended Glucantime regimen accordingly to the Brazilian Ministry of Health. In this presentation we will show our recent findings on the contribution of parasite's proteomics to the phenotype of refractoriness to antimony. First, we observed that poor treatment response is correlated with increased Sb^{III} IC_{50} values *in vitro*. In addition, we showed that Sb-responsive strain exhibits lower parasite counts and lower proportion of metacyclic-like forms than refractory strain. Second, label-free quantitative mass-spectrometry-based proteomics analyses revealed intrinsic differences at molecular level between those strains. Interestingly, protein concentration values clearly differentiated Sb-responsive from Sb-refractory parasites. In agreement with the increased growth profile *in vitro*, we observed that Sb-refractory parasites have a significantly higher cumulative concentration of proteins involved in DNA replication and RNA translation. Antimonial-refractory parasites also exhibit a significant increase in redox homeostasis and response to oxidative stress including increased abundance of trypanothione reductase, ascorbate peroxidase, tryparedoxin, and superoxide dismutase. Remarkably, we observed a significant increase in the abundance of proteins involved in amino acid metabolism parallel to a decrease in a series of membrane transporters. Although we are aware that cultured parasites do not exactly represent the

original population in patients' lesions, our results indicate that in the case of *L. guyanensis* there seems to be a good correlation between therapeutic response and *in vitro* susceptibility to antimony. Furthermore, *L. guyanensis* strains analyzed here have proteomics characteristics that indicate that the parasite may contribute to a proper or poor therapeutic response in patients undergoing treatment with pentavalent antimony.

S16-03: DRUG RESISTANCE IN *Leishmania*: DOES IT REALLY MATTER?

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Treatment failure (TF) jeopardizes clinical management of leishmaniasis and may be a major obstacle to disease control programs. It can be due to different factors related to the host, the drug, concomitant diseases and the parasite itself. From the parasite point-of-view, drug resistance (DR) is generally considered as the main actor of TF. In the past, we studied -in different epidemiological settings, parasite species and drugs- the link between TF and DR as measured by in vitro drug susceptibility assays. For both antimonials and miltefosine, we found a poor relationship between treatment outcome and drug susceptibility. At the light of recent findings in our research unit, we revisited these results and proposed different possible explanations to these discrepancies. In this presentation, we will aim to answer 3 fundamental questions. First, were the right assays used to measure DR? To answer this question, features and limitations of in vitro susceptibility assays will be discussed and contrasted with the contribution of molecular assays, especially whole genome sequencing. Secondly, were the right parasites studied? Drug susceptibility is measured on isolates and a strong emphasis will be made on the biases associated with the bottleneck of isolation and further selection in culture. Accordingly, parasites most fit in culture are not necessarily the same as the ones dominating in the patient and causing TF: this major bias was demonstrated when comparing parasite genome directly sequenced in patient's tissues and in derived cultivated isolates. Heterogeneity of parasite populations and an unprecedented level of aneuploidy mosaicism further complicate molecular tracking of DR. Thirdly, are there other parasite factors that may cause TF without DR? The case of co-infection with the endosymbiotic LRV will be briefly addressed but a major focus will be brought on parasite quiescence, a physiological stage of non-replication and strong metabolic modulation which allows *Leishmania* to survive to stress. Recent findings on the contribution of

quiescence to drug tolerance will be presented and the major conceptual difference with DR will be highlighted. In answer to the question of the provocative title, DR probably matters, but: (i) its impact on TF is not studied correctly (isolate = bias), (ii) molecular assays are likely more performant to track DR than in vitro susceptibility tests, but they should be applied directly in host's tissue for better predictability of treatment outcome, (iii) parasite heterogeneity and mosaicism complicate the molecular tracking of DR and (iv) parasite quiescence could cause TF without involving DR. Priority for further research will be discussed.

S17. VL ELIMINATION AS A PUBLIC HEALTH PROBLEM IN INDIA

S17-01: RETROSPECTIVE OVERVIEW OF THE VL ELIMINATION PROGRAM AND ADAPTIVE STRATEGIES FOR THE NEXT PHASE

Sridhar Srikantiah

CARE India

The VL elimination program in India gathered momentum around 2008 with the World Bank support ensuring additional program resources to modernise diagnosis and treatment and strengthened supervision of field operations. Although elimination deadlines were missed along the way, there was steady progress on most program coverage and impact parameters thereafter, with the expected resurgence to the next peak failing to materialise. We combine data and field observations from close quarters to analyse how the main interventions performed over time and why, covering IRS, case detection, information systems and allied health systems. Further, we elucidate lessons from these analyses and program insights to propose adaptations to the program strategy going ahead, with a view to achieve a meaningful and sustainable steady state of interventions in the program, along with preparedness for an uncertain future of the disease in currently endemic areas.

S17-02: DIAGNOSIS OF VISCERAL LEISHMANIASIS IN AN ELIMINATION SETTING: A VALIDATION STUDY OF THE DIAGNOSTIC ALGORITHM IN INDIA

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Visceral leishmaniasis (VL) is on the verge of elimination on the Indian subcontinent. Nonetheless, the current low VL-incidence setting brings along new challenges, one of which is the validity of the diagnostic algorithm, based on a combination of suggestive clinical symptoms in combination with a positive rK39 Rapid Diagnostic Test (RDT). With this study, we aimed to assess the positive predictive value of the diagnostic algorithm in the current low-endemic setting in India, by re-assessing the newly diagnosed VL patients with qPCR on venous blood as the reference test. In addition, we evaluated the specificity of the rK39 RDT, by testing apparently healthy individuals (without clinical VL) with the rK39 RDT. Participants were recruited in Bihar and Uttar Pradesh, India. VL patients diagnosed based on the diagnostic algorithm were recruited through six Primary Health care Centers (PHCs); non-VL cases were identified through a door-to-door survey in currently endemic, previously endemic, and non-endemic clusters, and tested with rK39 RDT, as well as, if positive , with qPCR on peripheral blood. We found that 95% (70/74; 95% CI 87-99%) of incident VL cases diagnosed at the PHC level using the current diagnostic algorithm were confirmed by qPCR. Among 15 424 apparently healthy

(without clinical VL) individuals, 39 were rK39 RDT positive, reflecting a specificity of the test of 99.7% (95% CI 99.7 – 99.8%). The current diagnostic algorithm combining suggestive clinical features with a positive rK39 RDT still seems valid in the current low endemic setting in India.

Keywords VISCERAL LEISHMANIASIS; DIAGNOSTIC ALGORITHM; rK39 RAPID DIAGNOSTIC TEST; qPCR

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S17-03: IS MOLECULAR XENOMONITORING A USEFUL TOOL FOR MONITORING VISCERAL LEISHMANIASIS TRANSMISSION IN THE PERI-ELIMINATION PHASE?

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The numbers of VL cases across the Indian Subcontinent (ISC) have decreased markedly as a result of interventions implemented during the regional kala-azar elimination programme. Consequently, active case detection is not a cost-effective surveillance approach moving forward and more sensitive surveillance approaches are required. For other vector-borne diseases targeted for elimination, such as lymphatic filariasis (LF) and onchocerciasis, molecular xenomonitoring (MX), detection of pathogen DNA/RNA, has served as a proxy of human infection to complement disease surveillance activities. The primary objective of the MX study was to investigate whether MX could be used to define endpoints of VL transmission and play a role in post-elimination surveillance of VL (similar to using MX in the LF elimination programme to monitor residual transmission post-MDA). To calculate these endpoints requires estimates of the *L. donovani* DNA infection and infectiousness rates in *Phlebotomus argentipes* females, the only know vector of VL in ISC, to be made and compared with VL infection rates in humans. The study was conducted in 12 villages (endemic, previously endemic and non endemic) located in 5 districts in Bihar. Using previous infection data, a sample size calculation was performed and a target of 3,750 *P. argentipes* females was required to detect differences in *L. donovani* DNA prevalence rates between treatment groups (levels of endemicity). Following a significant sampling effort undertaken between 2018-2021, which was interrupted by the COVID-19 pandemic and local flooding, we collected over 3,900 *P. argentipes* females for *L. donovani* screening. Each female sandfly was dissected at the upper

thorax in our attempts to quantify sandfly infection (using pools of lower thorax-abdomen sections) and infectiousness (using individual head/thorax sections) rates for *L. donovani* DNA. Prior to screening, 10 real time PCR assays, targeting different genes of the *L. donovani* genome, were tested to evaluate sensitivity and specificity. Most of these published protocols were developed specifically for detecting *L. donovani* in human patient samples. When used to detect *L. donovani* in sandflies, we found that some PCR targets cross reacted to either sandfly DNA, or to the DNA of non target pathogens, so were not fit for purpose. We optimized two protocols that were specific for *L. donovani* DNA detection in sandflies. DNA extractions were performed on 172 pools of lower thorax-abdomen sections of between 4-14 sandflies/pool (pooled by collection date and village). Pools were analysed for the presence of *L. donovani* DNA using a qPCR protocol with Taqman primers and probes. Samples were considered positive if Ct values were lower than the limit of detection by the assay (Ct<31). All samples and controls were run in duplicate. Sequencing was performed on any pools that were preliminary considered to be positive for *L. donovani*. Following molecular analyses, none of the pools were confirmed as positive for *L. donovani* DNA. The effort required to collect large numbers of female *P. argentipes* for *L. donovani* detection, during this period of low endemicity, was challenging and resource demanding. In conclusion, establishing a relationship between *Leishmania* infection rates in human and sandfly populations may not be feasible in elimination scenarios. Although RT-PCR may be considered the 'gold standard' for detecting pathogen DNA by researchers, it is an unrealistic approach for programmatic use when infection rates in vectors are so very low (0-0.03% estimates in Bihar at this time). We recommend that to optimise the use of limited programmatic resources, new field-friendly tools are required with protocols for simultaneous detection of *Leishmania* and other pathogens present in sandfly and mosquito populations. To address this need, we have developed a point-of-need multiplex tool for screening sandflies and mosquitoes for a range of pathogens, bloodmeal sources and for insecticide resistance mutations. This isothermal recombinase polymerase amplification test (RPA), is a highly sensitive and selective isothermal amplification technique, operating at 37–42°C, with minimal sample preparation and capable of amplifying as low as 1–10 DNA target copies in less than 20 min. It is

therefore more suitable than other methods for field use. The multiplex tool requires field validation but, when combined with a mobile data management system to facilitate timely data reporting, may offer a method for rapidly screening all blood-fed sandflies and mosquitoes in a collection to determine which pathogens are circulating in an area of concern. An integrated vector surveillance approach may be the most feasible path forward in vector-borne disease control and elimination efforts.

Keywords MOLECULAR XENOMONITORING; *Leishmania donovani*; ELIMINATION, MULTIPLEX TOOL; INTEGRATED VECTOR SURVEILLANCE

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S18. VECTOR COMPETENCE AND *Leishmania*-SAND FLY INTERACTIONS

S18-01: *Leishmania* CO-OPTS IGM NATURAL ANTIBODIES PREVALENT IN VERTEBRATE BLOOD FOR GENETIC EXCHANGE IN SAND FLIES

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The critical role of multiple blood meals on *Leishmania* establishment, differentiation and sand fly infectioussness has been recently established. Here we demonstrate that blood feeding of sand flies has an equally profound outcome on *Leishmania* genetic exchange. *Leishmania* genetic

exchange occurs inside the gut of the vector sand fly and has been shown to occur via intraspecies and interspecies combinations. Recently, genetic exchange was achieved in vitro with several *Leishmania spp.,* particularly *L. tropica,* but failed with *L. major*. Here, we discovered that *L. major* hybrid formation in vitro requires IgM, an evolutionary conserved natural antibody in adult serum. IgM facilitates the formation of a "leishmania mating clump (LMC)", a transient spherical structure formed by an aggregation of live parasites. The LMC formed with sera from 14 different animals and was observed in all of the 8 tested *Leishmania* species. Electron microscopy revealed that the LMC sustains close interactions between parasites that promotes parasite fusion and genetic exchange. In vivo, IgM was essential for *Leishmania* hybrid formation in two of three parasite lines used in parental combinations; in the third combination, IgM increased by 12 folds the number of flies with *Leishmania* hybrids. Most importantly, second generation hybrids, produced by backcrossing hybrid and parental lines, were reproducibly recovered from sand flies but only in the presence of IgM. Collectively, these data demonstrate that *Leishmania* exploits IgM, present in blood of all vertebrates, and the natural feeding behavior of its insect vector to promote genetic exchange.

S18-02: LESSONS LEARNED FROM EXPERIMENTAL *Leishmania* HYBRIDIZATION

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By transmitting hundreds of pathogens causing important human mortality and morbidity, vector insects are a major public health threat. Even if intrinsic insect parameters such as their immune response are known to impact their vectorial capacities, the vector/pathogen interaction is still little understood. Phlebotomine sand flies are responsible for the transmission of *Leishmania* parasites, the causative agent of Leishmaniasis. In addition to their clonal reproduction, *Leishmania* can engage in a cryptic sexual cycle resulting in the production of hybrid progeny. Hybridization is believed to be a major source of *Leishmania* genetic diversity, which drives changes in tissue tropism, pathology and drug resistance. Experimental generation of hybrids was for long confined to parasites growing in the sand fly gut, which is associated with an inherent difficulty to identify and observe the process of hybridization and its underlying molecular mechanisms. We developed a protocol enabling the reproducible production of *Leishmania* hybrids *in vitro*, independently of the use of their sand fly hosts. However, despite the successful generation of hybrids in axenic cultures, the frequency of hybrids generated *in vivo* is much higher than *in vitro*, showing that the vector gut environment is particularly adapted for *Leishmania* sexual mating. The mechanisms of *Leishmania* hybrid production and the parameters rendering the vector gut favorable to this process are currently poorly understood. We recently showed that culture conditions inducing genotoxic stress, such as exposure to X-irradiation, to Reactive Oxygen species (ROS) or to the alkylating agent methyl methanesulfonate (MMS),

leads to a drastic increase in *Leishmania* hybrid production *in vitro*, suggesting a link between DNA damage repair and hybridization. Following the idea that stress-exposed cultures of *Leishmania* should contain a higher proportion of mating competent cells than untreated cultures, we established the single cell transcriptome of *Leishmania* cultures exposed or not to irradiation to identify hybridization-competent parasites and establish their gene expression profile. This approach identified clusters of cells enriched in irradiated cultures and marked by the expression of genes involved in meiosis or DNA damage repair in other organisms, such as the ancestral gamete-fusogen Hap2, the nuclear-fusion agent Gex1 or the DNA recombinase Rad51. Using transgenic tagging of the endogenous Hap2 protein with a fluorescent marker followed by cell sorting of fluorescent parasites by FACS, we could show that Hap2 protein expression correlates with *Leishmania* hybridization frequency. In addition, unpublished results show that Gex1 null mutant strains failed to produce hybrids *in vitro*, suggesting an essential role for this protein in *Leishmania* hybridization. In conclusion, we established an in vitro system to investigate the mechanisms controlling *Leishmania* hybridization, which allowed us to identify genes involved in the production of hybrids, such as Hap2 and Gex1, and parameters that could influence hybrid production, such as DNA damaging conditions. Future challenges lie in describing the precise mode of action of Hap2 and Gex1 in hybridization, and analyzing the mechanistic link between DNA damage repair and hybrid formation.

S18-03: EXPERIMENTAL TRANSMISSION OF *MUNDINIA* AND *PORCISIA* BY INSECT VECTORS

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Leishmania parasites are currently divided into four subgenera: *Leishmania*, *Viannia*, *Sauroleishmania* and *Mundinia*. The recently established subgenus *Mundinia* has a wide geographical distribution and contains five species, three of which have the potential to infect humans. The genus *Porcisia* accommodates two species, dixenous parasites of Neotropical porcupines, originally described as *Leishmania hertigi* and *L. deanei*. While *Leishmania*, *Viannia* and *Sauroleishmania* are transmitted exclusively by phlebotomine sand flies (Diptera: Psychodidae), natural vectors of *Mundinia* and *Porcisia* remain uncertain. This study investigates the potential of sand flies and biting midges of the genus Culicoides (Diptera: Ceratopogonidae) to transmit these parasites. Insects were exposed to parasites through a chicken skin membrane and dissected at various time intervals post bloodmeal. Potentially infected females were also allowed to feed on ear pinnae of anaesthetized BALB/c mice and the presence of parasite DNA was subsequently confirmed in the mice using qPCR. All *Mundinia* species tested developed better in *C. sonorensis* than in sand flies: they were able to establish infection at a high rate, successfully colonize the stomodeal valve and produce a higher proportion of metacyclic forms. Subsequently, three

parasite species, *L. martiniquensis*, *L. orientalis* and *L.* sp. from Ghana, were transmitted to the host mouse ear by *C. sonorensis* bite while transmission experiments entirely failed with *P. argentipes*. *Porcisia hertigi* did not survive defecation in *L. longipalpis* and *L. migonei* females. On the other hand, *P. deanei* colonized Malpighian tubes of *L. longipalpis* and was transmitted on BALB/c mice by prediuresis of blood feeding females. This type of development and transmission route is unique among trypanosomatids. Our results also provide the first detailed in vivo evidence that biting midges can act as competent vectors of the subgenus *Mundinia* that has considerable epidemiological implications for control of these neglected pathogens.

Keywords *Mundinia*, *Porcisia*, *Phlebotomus*, *Lutzomyia*, *Culicoides*

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S18-04: SUSTAINED INFECTION OF NEW WORLD SANDFLY VECTORS FOR DIFFERENT *Leishmania* SPECIES

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In the new world, some species of sandflies are renowned as primary vectors. Such as:
Lutzomyia longipalpis to *Leishmania infantum, Lutzomyia intermedia,* and *Lutzomyia migonei* to *Leishmania braziliensis*, but the role of those flies as a competent vector for other protozoan is unclear. Laboratory and field findings have contributed to the hypothesis of permissiveness. Here we evaluate vector competence of *Lu. longipalpis*, *Lu. intermedia, and Lu. migonei* upon infection with different species of *Leishmania*. We compared infection intensity with different concentration of parasites, their location in the midgut, metacyclogenesis process, and the transmission by sandfly bite effectiveness. *Lu. longipalpis* was able to sustain infection of all species of *Leishmania* at the high parasite dose, but its permissiveness is dose-dependent. *Lu. intermedia* only maintain a mature infection with *L. braziliensis. Lu migonei* was able to sustain the infection to all parasites tested, and the highest parasite infection was with *L. chagasi* and *L. amazonensis*. The transmission by the sandfly bite showed that the *Lu. longipalpis* is a competent vector of *L. chagasi* and *L. major,* and *Lu migonei* of *L. chagasi* and *L. braziliensis* parasites. However, these studies indicate the permissibility of other parasites. The Laboratory permissibility can be dose-dependent, which may explain infection in the field. Supporting that *Lu*

longipalpis and *Lu migonei* play a fundamental role in the epidemiology of visceral leishmaniasis in Brazil and Argentina.

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S19. DRUG TARGET IDENTIFICATION

S19-01: CURRENT STATUS AND FUTURE OPPORTUNITIES FOR DRUG DISCOVERY FOR LEISHMANIASIS

Charles E. Mowbray

Dugs for Neglected Diseases initiative (DNDi)

Significant advances in drug discovery for leishmaniasis have been made in the last decade. A review of the current status and research landscape will highlight (i) the opportunities presented by recently discovered drug candidates, (ii) the remaining unmet needs, and (iii) identify opportunities for future work. The properties of the new chemical entities in development for leishmaniasis will be reviewed. Recent progress in characterizing the mechanism of action (MoA) of these drug candidates provides valuable information to guide their development and possible use in combinations. An understanding of the relative stages of development of new drugs and whether they will allow testing of the associated mechanisms of action in clinical studies provides useful guidance to leishmaniasis drug discovery teams as they seek to identify further drug candidates to strengthen and complement the global portfolio. Discovery of further drug candidates remains an important objective. Current and future drug discovery strategies might include (i) discovery of back-up candidates with previously identified antileishmanial MoA, (ii) discovery of protypes with novel antiparasitic MoA, (iii) exploration of host directed therapies to inhibit parasite replication or survival or to modulate the immune response to infection.

Keywords LEISHMANIASIS; DRUG DISCOVERY

S19-02: A STRUCTURE-BASED DRUG DISCOVERY PROGRAM TARGETING *Leishmania* GLYCOGEN SYNTHASE KINASE 3

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Leishmaniasis is a complex vector-borne disease caused by the protozoan parasite *Leishmania spp*. Infection with parasites results in diverse clinical manifestations, ranging from localized skin ulcers in cutaneous leishmaniasis to potentially fatal systemic disease in visceral leishmaniasis. The disease has strong links with poverty, with over a million estimated new cases per year worldwide. Despite its large health and social impact, leishmaniasis is not seen as a priority by the pharmaceutical community and policymakers, it thereby remains a truly neglected tropical disease. Drugs currently available are toxic, antiquated, not adapted to the field, and show variable efficacy, there is, therefore, a clear need for new treatment options. Phenotypic-based drug discovery campaigns allowed an unprecedented portfolio of new chemical agents to recently progress to clinical evaluation. However, the development of new drugs to treat leishmaniasis has been hampered by the difficulty of identifying valid therapeutic targets. Protein kinases are promising candidates for drug development against parasitic diseases. The long and short isoforms of *Leishmania* glycogen synthase kinase 3 (GSK3a/b, respectively), a multifunctional Ser/Thr kinase, have been identified as genetically essential for parasite viability and have been pharmacologically explored to some extent. In this work the team reports

efforts within a Latin American Open-Science target-based drug discovery program to develop potent and selective inhibitors of *Leishmania infantum* GSK3a and GSK3b. Recombinant protein kinases were produced and screened against a compound library obtained from commercial sources. Biochemical assays were employed to validate positive hits and to determine IC_{50} and Ki values. Promising compounds (active in the nanomolar range) were further investigated by cellular assays to evaluate antileishmanial activity and host cell toxicity. Additional data was generated to support the prioritization of chemical scaffolds, such as selectivity over a panel of human kinases and ADME properties. The team is currently analyzing data obtained from protein-inhibitor complexes to elucidate ligand-binding modes and to guide the design of optimized analogues for two different chemical series. The goal of the current project stage is to enable the progression of lead compounds to proof-of-concept studies in animal models of the disease.

Keywords *Leishmania*; DRUG DISCOVERY; DRUG TARGET; GSK3.

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S19-03: THE USE OF MULTIPLE ORTHOGONAL METHODOLOGIES TO DETERMINE THE MECHANISM OF ACTION OF PROMISING ANTI-LEISHMANIAL COMPOUNDS".

Marta Lopes Lima

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A severe lack of robustly validated drug targets has proved a barrier to target-focused drug discovery for many neglected tropical diseases, including leishmaniasis. This has left drug discovery programs heavily reliant upon phenotypic screening to identify chemical start points. Although successful in many aspects, phenotypic screens do not provide information regarding the mechanism(s) of action and/or molecular target(s) of the active compound. Such knowledge is crucial in the development and optimization of a chemical series and is often invaluable in the development of strategies to circumvent issues such as poor pharmacokinetic properties and toxicity. Comprehensive drug target deconvolution studies, often time-consuming, are best achieved by employing a range of strategies allowing the robust identification and validation of molecular targets. With this in mind, at the Mode of Action Group, University of Dundee we have assembled a platform of orthogonal approaches in the fields of high-throughput genetics, cell biology and biochemistry, and chemical proteomics to facilitate drug target deconvolution studies in neglected tropical diseases. Here, I will detail our mode of action workflow, outlining the advantages and disadvantages of the various methodologies we utilise. I will then describe how this powerful combination of approaches led to the identification of oxidosqualene cyclase (OSC), a key enzyme of sterol biosynthesis, as the target of a benzothiophene compound demonstrating promising anti-leishmanial activity.

S19-04: THE MACROPHAGE LYSINE DEMETHYLASE LSD1 AS A POTENTIAL TARGET FOR HOST-DIRECTED ANTILEISHMANIAL DRUG DISCOVERY

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Leishmania parasites develop as strictly intracellular amastigotes, mainly inside macrophages. These parasites have evolved sophisticated strategies to escape host cell defense mechanisms and to hijack cellular processes for their benefit. The control of these parasites in humans relies exclusively on chemotherapy. The rise of treatment failure observed for current anti-leishmanial drugs call for the discovery of new treatment options that are more refractory to drug resistance. Here we propose host-directed therapy as such a novel strategy. Indeed, the strict dependence of intracellular *Leishmania* survival on macrophage physiology and biology proposes the host cell as well as essential pathways of host/parasite interaction as interesting targets for anti-leishmanial drug discovery. Applying phenotypic and biochemical screening assays, we previously discovered a series of mammalian epigenetic inhibitors that show a host-dependent profile as they significantly decrease the intracellular parasite load but not extracellular *Leishmania amazonensis* parasites. Two of the identified hits were designed chemically to inhibit mammalian Lysine-specific demethylase 1 (LSD1), a known driver of tumor development and a key regulator involved in

macrophage polarization. This epigenetic eraser enzyme removes methyl groups from mono- and di-methylated lysine 4 and 9 on histone H3, thus acting both as a repressor and activator of gene expression, respectively. We first validated the activity of the LSD1 inhibitors (LSD1*i*) in primary mouse macrophages and RAW 264.7 cells infected with *L. amazonensis*. We showed that both LSD1*i* killed intracellular amastigotes but not extracellular promastigotes at concentrations ranging from 1-10 µM, confirming their host-directed mode of action. We next validated the inhibitor effect on LSD1 using an *in vitro* activity assay, and established LSD1*i* specificity applying a Cellular Thermal Shift Assay (CETSA), which revealed that LSD1*i* thermally stabilizes host LSD1, but not related LSD2 and monoamine oxidase A, or the negative control protein GAPDH. Surprisingly, LSD1 thermal stabilization was also observed by infection alone, suggesting that parasites may affect LSD1 interactions and alter the composition of LSD1 complexes. Finally, we analyzed changes in gene expression and protein abundance by RNA-seq and WB analyses of infected and LSD1*i*-treated macrophages. While *Leishmania* infection neither altered LSD1 expression nor nuclear localization, intracellular parasites inhibited macrophage innate and pro-inflammatory pathways and caused transcriptional up-regulation of the cholesterol biosynthesis pathway, both of which may contribute to intracellular parasite survival. Indeed, treatment with LSD1*i* counteracted these macrophage expression changes and largely restored their normal immuno-metabolic functions, including rescue of the anti-microbial response and reversion of sterol biosynthesis to levels observed in uninfected cells, which correlated to reduced parasite burden. In conclusion, we identified macrophage LSD1 as a host-directed drug target and revealed two LSD1 inhibitors as specific drug candidates for host-directed, anti-leishmanial therapy. CRISPR/Cas9-mediated ablation of LSD1 in primary macrophages, the analysis of LSD1 complexes, and ChIPseq profiling of LSD1 promoter occupancy and LSD1-mediated histone modification in infected and LSD1*i* treated macrophages will provide us important mechanistic insight into the role of LSD1 in intracellular *Leishmania* survival and the mode of LSD1*i* drug action in primary macrophages.

Keywords MACROPHAGE, *Leishmania*, EPIGENETICS, LYSINE SPECIFIC DEMETHYLASE 1

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S19-05: KINETOPLASTID DRUG DISCOVERY AIDED BY COMPUTATIONAL SCIENCE AND ARTIFICIAL INTELLIGENCE

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Kinetoplastid diseases encompass Leishmaniasis, Chagas disease and Sleeping Sickness. Together these diseases threaten over a billion people living in tropical and sub-tropical regions worldwide. These diseases cause substantial deaths and morbidities in millions of people. Current drugs suffer from inadequate efficacies and toxicities. There is an urgent need to develop novel, direct, anti-parasitic treatments that are efficacious, safe and with shorter treatment regimen. Over the last decade computational science and artificial intelligence (AI) have revolutionized various aspects of day-to-day life. Here, we describe use of these technologies in advancing kinetoplastid drug discovery. Computational science and AI aided by machine learning, have facilitated many stages in drug discovery, from selection of libraries for screening to designing novel molecules with better physico-chemical properties and potential for lower toxicities. The massively multi-task pQSAR models, combining 13,000 in-house biological assays, helped in evaluating potential growth inhibition and off-target liabilities of novel molecules. Proteasome and CLK1 targets were shown to be pan-kinetoplastid targets having potential for use against both Leishmaniasis and Chagas disease. Computational homology modeling of proteasome and CLK1 targets not only predicted the structure accurately, but also helped in progressing medicinal chemistry optimization of compounds before the structure data was deciphered. We are also efficiently adopting de-novo generation of novel phenotypically-active molecules from biological signatures using AI driven generative chemistry.

S20. LEISHMANIASIS VACCINE: PAST, PRESENT AND FUTURE

S20-01: PROGRESS TOWARDS DEVELOPING A SAFE AND EFFICACIOUS PAN *Leishmania* VACCINE

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Leishmaniasis is a neglected tropical disease with significant morbidity and mortality. Currently, there is a lack of an effective strategy to control this disease and achieve elimination by 2030, the target set forth by WHO. Vaccination can be an effective measure to control this disease and has the potential to achieve its elimination. Previously, efforts focused on the development of vaccines from killed parasites with or without adjuvants, subunit vaccines, and DNA/RNA based vaccines that were immunogenic and efficacious in animal models against virulent infection through needle

injection. However, when some of them were tested against natural sand fly infection or in clinical settings, they were determined not to be efficacious. People who have recovered from leishmaniasis are protected for life against future infections, suggesting that an effective vaccine is possible against this disease. Exposure to, or deliberate infection with wild type *Leishmania major* (leishmanization), was shown to be effective against both visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL). However, the practice of deliberate infection with the wild type parasites raises safety concerns and has not been pursued. Therefore, a well characterized genetically modified parasite that is safe, but equally efficacious as leishmanization, could be an alternative. The presence of both CL and VL in many of the endemic countries makes it desirable to have a vaccine that can protect against both forms of the disease. We have developed a vaccine candidate that has been genetically modified by deleting centrin gene, an essential gene for cell division for amastigotes, using CRISPR-Cas technology in a dermotropic *Leishmania major* parasite strain (*LmCen-/-*). We have shown that immunization with *LmCen-/-* parasites is safe and efficacious in appropriate animal models for both CL and VL against both needle and sand fly vector mediated virulent infection. Additionally, in animal models of CL, we have demonstrated that immunization with *LmCen-/-* induces effector and memory T cell responses comparable to leishmanization and equivalent protection against virulent infection. Further, the *LmCen-/* parasite vaccine manufactured in a bioreactor under Good Laboratory Practice, is safe, immunogenic, and provides long-term protection against both CL and VL in sand fly vector mediated challenge in preclinical studies. We have also demonstrated that GLP grade *LmCen*$^{-/-}$ parasites can elicit pro-inflammatory immune responses in peripheral blood mononuclear cells isolated from healthy individuals from nonendemic as well as from healthy, asymptomatic and VL healed individuals from endemic region suggesting that *LmCen-/-* parasite vaccine has the potential to induce a protective immune response in humans. In addition, we are developing biomarker of correlates of protection for *Lmcen-/-* parasites using *Leishmania donovani* total antigens under cGMP for *Leishmania* Skin Test that could be used in clinical trials. Currently, the *LmCen-/-* parasite vaccine is being manufactured under cGMP conditions for future clinical trials. Taken together, these

results suggest that a genetically modified parasite could be a viable vaccine candidate for evaluation in clinical trials.

Keywords PAN *Leishmania* LIVE ATTENUATED VACCINE; SAFE AND EFFICACIOUS; SANDFLY CHALLENGE; CGMP MANUFACTURING

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Communication Disclaimer: My contributions are informal communication and represents my own best judgement. These comments do not bind or obligate FDA.

S20-02: NEOLEISH, FIRST NAKED DNA PLASMID VACCINE AGAINST *Leishmania infantum* IN DOGS

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Neoleish® is a needle-free DNA plasmid vaccine candidate. It contains the nucleotide sequence of LACK protein from *Leishmania infantum* and no antibiotic resistance marker. The pharmaceutical form is a solution for intranasal administration, no adjuvant is added. Primary vaccination course consists of two doses of 1 ml, 2 weeks apart. Data from stability studies support a shelf-life of 24 months stored at +2°C - +8°C. The vaccine has been developed following the requirement of the current European legislation on DNA Veterinary vaccines. Its safety was evaluated in Beagle dogs. The following controlled studies were conducted: *(i)* safety of the administration of one dose and a repeated dose, *(ii)* safety of the administration of three doses, *(iii)* safety of the administration of an overdose and the administration of a repeated dose and *(iv)* biodistribution, chromosomal integration, excretion and reproductive toxicity. Efficacy against *L. infantum* was tested in dogs by experimental challenge and in naturally infected under field conditions. For laboratory studies, a challenge model was developed. The circulating IgG1/IgG2/total IgG levels were quantified by ELISA. Cellular immune response (CD4+ lymphoproliferation assay against CLA and LACK proteins, cytokine quantification of INF-γ and IL-10 specific release) was evaluated at PBMC level and in relevant tissues (spleen, lymph node and liver). The efficacy was measured by clinical signs evaluation and parasite burden quantification. Parasite load was measured by means of

qPCR in samples of blood and bone marrow, at different times post infection, and in relevant tissues when the studies finished (one year after challenge). Experimental infections were performed at different times post-vaccination to establish onset of immunity and duration of immunity. Finally, a GCP-compliant safety and efficacy field trial was conducted between 2017 and 2019. 361 dogs were enrolled from 8 kennels located in 3 Spanish regions. Neoleish® showed a high safety profile: no temperature increases and no local or systemic reaction were detected. In the biodistribution study plasmid was detected on nasal mucosa, local lymph nodes and faeces. Reproductive tissues were negative. No integration was observed. The vaccine doesn't interfere with the reference diagnostic test (IFA). In field conditions, the safety profile of the vaccine was confirmed. The vaccine induces stimulation of the cellular immune response, both post vaccination and post-infection, in blood and relevant tissues. Vaccination was associated to *(i)* significant reduction of parasite burden in bone marrow and blood (with potential impact in transmission), *(ii)* significant reduction of number of dogs infected, *(iii)* significant reduction of clinical signs and *(iv)* reduction in the number of symptomatic dogs. In conclusion, Neoleish® is a safe DNA vaccine that has shown efficacy against *Leishmania infantum,* based on reduction of the parasite load and clinical signs, either in experimental and natural conditions.

Keywords DNA PLASMID VACCINE; LACK; *Leishmania infantum*

S20-03: DEVELOPMENT OF A cGMP GRADE PROCESS TO PRODUCE LIVE ATTENUATED *Leishmania* PARASITES AS A PAN-*Leishmania* VACCINE

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Leishmaniasis is a neglected tropical disease responsible for causing about 1.7 million new infections every year. A prophylactic vaccine will be an effective method of protection against leishmaniasis, reducing the transmission and supporting the elimination of the disease globally. Unlike other infections, the patient who recovers from leishmaniasis develops immunity against reinfection, thus vaccination could be a viable approach to acquire protective immunity. Preclinical studies with a genetically attenuated *Leishmania major* centrin deletion mutant (*LmCen*$^{-/-}$) showed excellent protection against sand fly mediated challenge with *Leishmania major* and *Leishmania donovani* parasites indicating that *LmCen*$^{-/-}$ parasites could be tested as pan-*Leishmania* vaccines in clinical trials. Towards this goal, we developed a scalable cGMP process, through which we have grown *LmCen*$^{-/-}$ strain from 1L to 5L, 10L, and 20L and demonstrated the scalability and robustness of the production process. Parasites produced from the bioreactor have been characterized using different parameters, specifically its in-vitro infectivity and protection in the animal challenge model. Currently the *LmCen*$^{-/-}$ vaccine preparation produced under necessary regulatory guidelines is going through preclinical toxicity studies before proceeding to Phase-I clinical trials to assess safety and immunogenicity characteristics of this vaccine.

Keywords *Leishmania*, CENTRIN; VACCINE; cGMP; BIOREACTOR

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S20-04: IMMUNIZATION WITH CENTRIN-DEFICIENT *Leishmania braziliensis* DOES NOT CONFER PROTECTION AGAINST SUBSEQUENT INFECTION

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Leishmaniasis affects millions of people in major areas of the globe. Despite this disease burden, a vaccine remains unavailable. Previous infection by *Leishmania* induces robust immunity against subsequent disease, indicating that vaccination is an attainable goal. Recent advances in this field have shown that immunization with *Leishmania* lacking *Centrin* confers robust protection against challenge. *Centrin* is a calcium-binding cytoskeletal protein involved in centrosome duplication. Centrin deficiency in *Leishmania* causes arrested division at the amastigote stage, resulting in an attenuated cell line, unable to cause disease upon experimental infection. Herein, we employed the LeishGEdit toolbox to generate a Centrin-deficient *Leishmania braziliensis*, the causative agent of mucosal and disseminated disease. Firstly, we generated a transgenic cell line expressing Cas9 and T7 RNA polymerase, which was later employed for the targeted deletion of Centrin. Whole-genome sequencing of centrin-deficient *L. braziliensis (LbCen$^{-/-}$)* did not indicate the presence of off-target mutations. *LbCen$^{-/-}$* promastigotes displayed normal *in vitro* growth whereas axenic and intracellular *LbCen$^{-/-}$* amastigotes showed a multinucleated phenotype with impaired survival following macrophage infection. Upon experimental infection of BALB/c mice with *LbCen$^{-/-}$* promastigotes, lesions failed to develop whereas parasites were detected two weeks later at the inoculation site. Parasites became undetectable after five weeks of infection, indicating impaired survival of *LbCen$^{-/-}$ in vivo*. Surprisingly, mice immunized with

LbCen$^{-/-}$ and challenged with *LbWT* parasites were not protected as lesion development and parasite multiplication were observed. On the contrary, mice that healed a primary infection with wild-type *L. braziliensis* were successfully protected, failing to develop lesions and displaying a significantly lower parasite load, compared to control mice. These results indicate that immunization with the attenuated *LbCen*$^{-/-}$ does not protect against a challenge infection, differently from results reported for other species. We hypothesize that the effector immune response induced by *LbCen*$^{-/-}$ is not robust or adequate to prevent disease development. In conclusion, the complexity of the immune response against *Leishmania* sp. highlights differences regarding protective immune responses, and indicates that investigating these discrepancies shall contribute to advances in the field of vaccine development.

Keywords LeishGEedit; LEISHMANIASIS; GENETIC MANIPULATION; ATTENUATION; VACCINE DEVELOPMENT

S20-05: SINGLET OXYGEN-KILLED *Leishmania* ARE SAFE AND EFFECTIVE VACCINES AND VACCINE PLATFORMS

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Singlet oxygen ($^1O_2$) is a highly oxidative cytotoxic radical. Plant produces it abundantly during photosynthesis from light excitation of chlorophyll tetrapyrrole intermediates in the presence of atmospheric oxygen. Chloroplasts thus contain abundant carotene and tocopherol strategically placed to scavenge this radical effectively for detoxification. Non-photosynthetic heterotrophs, e.g. animals, *Leishmania* and other trypanosomes are not known to produce $^1O_2$, hence evolving no specific mechanism for its neutralization. They are thus highly sensitive to inactivation by $^1O_2$-mediated oxidation of cellular lipids, proteins and nucleic acids. The half-life of $^1O_2$ is extremely short (microseconds), making it most cytotoxic when generated endogenously in the cells. Loading of *in vitro* cultured promastigotes with photosensitizers, through chemical and genetic engineering strategies, allowed them to produce endogenous $^1O_2$ for rapid and complete cell inactivation on exposure to dim light (see Sections 3.6.2 in Reference [1]). The photosensitizers, phthalocyanines were chemically engineered during their synthesis with the addition of axial ligands to its coordinating metals (Si, Zn), rendering them hydrophilic and cationic to facilitate their uptake by *Leishmania* into the endosomes. *Leishmania* accumulate cytosolic photosensitizers when genetically engineered by exploiting their genetic deficiencies in porphyrin/heme biosynthesis. The genes coding for the first five of the eight enzyme cascades in this biosynthetic pathway are absent in the genomes of *Leishmania* spp. Promastigotes were thus transfected with mammalian cDNAs to express aminolevulinate dehydratase (ALAD) and porphobilinogen deaminase (PBGD), the 2nd and 3rd enzymes in this pathway. Exposure of these

transfectants to delta-aminolevulinate (ALA) – the product of the 1st enzyme, common to heme and chlorophyll biosynthesis, led to the production of water-soluble uroporphyrin 1 (URO-1), which accumulated to a very high level cytosolically due to the absence of the downstream URO-utilizing enzyme. Double loading of *Leishmania* with two different photosensitizers, i.e. a cationic phthahlocyanine in the endosomes and URO-1 in the cytosol, ensures full photosensitization of the cell population for complete $^1O_2$ mediated-inactivation initiated by light. The inactivated promastigotes cease their flagellar motility, but maintain their structural integrity. They fail to replicate *in vitro* as promastigotes in culture medium, to differentiate into amastigotes in macrophages and to produce lesions in mice. Singlet oxygen-killed *Leishmania* are immunogenic. They interact with macrophages to produce cytokines for immunity, unlike live parasites for immunosuppression *in vitro*. Collaborative work in colleagues' labs showed that vaccination of susceptible animals with $^1O_2$-lnactivated *Leishmania* protected them against experimental cutaneous and visceral leishmaniasis. The $^1O_2$-killed *Leishmania* is a potential platform for safe and effective delivery of vaccines to elicit T cell immunity against other diseases. Experimentation with *Leishmania* transfected to express ovalbumin (OVA) provided the initial evidence for this. Dendritic cells and macrophages loaded with $^1O_2$-killed OVA-expressing transfectants resulted in the co-presentation of OVA CD8 T cell epitope (SIINFEKL) and MHC Class I molecules. These primed antigen-presenting cells were able to activate the cognate epitope-specific CD8 T cells *in vitro*. The positive outcome with OVA led us to transfect *Leishmania* for expressing vaccine candidates of malignant and viral diseases, e.g. human enolase 1 (hENO1) for lung cancer. Immunization of lung cancer cell-implanted mice with $^1O_2$-killed hENO1-expressing *Leishmania* suppressed the emergence of otherwise massive tumors in the peritoneal cavity. Similar approaches of vaccination produced promising results against viral diseases in mouse models. These preliminary observations are worthy of further exploration.

Keywords SINGLET OXYGEN; INACTIVATED *Leishmania*; VACCINE; VACCINE PLATFORM; TUMOR IMMUNOTHERAPY

S21. NEW GUIDELINE FOR THE TREATMENT OF LEISHMANIASIS IN THE AMERICAS: WHAT HAS CHANGED?

S21-01: PAHO Therapeutic recommendations in cutaneous and mucosal leishmaniasis, 2022

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With nearly 40,000 new cases per year of cutaneous leishmaniasis (CL) and 2,000 of L. mucosa (ML), this disease has important public health repercussions in at least 18 countries of the Americas. Until a few years ago, first-line treatment was exclusively with parenteral pentavalent antimonials (PA) and the results were acceptably good in terms of safety and efficacy. But the loss of effectiveness in all the clinical forms but especially in the most severe ones such as disseminated cutaneous, diffuse cutaneous and mucosal forms, with frequent therapeutic failures and relapses, made it necessary to prolong the treatment in such a way that safety was compromised, forcing a search for new forms of management, either through the use of new medications, modifications in the routes and forms of administration of medications already in use, or by combining drugs. The contribution of evidence has been especially fruitful in the last fifteen years and especially in the last 9, since when PAHO published its Therapeutic Recommendations in 2013. In the new edition of the guidelines for the treatment of leishmaniasis in the Americas published last June, the updated recommendations for the management of the different forms of CL and ML are collected, taking into account the judicious evaluation of the published material with emphasis in controlled clinical studies, but also opening space

for discussion of the clinical experience of the panel of experts in those areas of knowledge that were critical but in which there were no clinical trials or sufficient published material, so that the health professionals could find indications for the situations that they must face on a day-to-day basis. It is noteworthy that these new guidelines for the treatment of leishmaniasis in the Americas bring about a substantial change in the role of PA for the management of localized cutaneous leishmaniasis, which accounts for around 80% of cases; In these patients -if they meet defined criteria- intralesional administration becomes the strongly recommended treatment, while its parenteral use is reserved for cases in which no other management option is possible. Likewise, oral miltefosine becomes a strong recommendation, that is, the first option for treatment in children and adults when the prevalent species are *L. panamensis*, *L. guyanensis* or *L. braziliensis* and even *L. mexicana* in adults. Parenteral pentamidine, thermotherapy, and paromomycin cream may be considered useful options if intralesional PA or oral miltefosine cannot be used for some reason. In addition to reducing the risks of adverse events, these new therapeutic recommendations seek to facilitate treatment both for those who apply it (health professionals) and for patients, thus seeking to reduce some of the conditions that limited access to therapy. For the management of CL, in special situations such as pregnant women, heart patients, liver or kidney patients, immunosuppressed, etc., there are specific indications. For ML, the evidence found supports the use of parenteral PA with or without pentoxifylline, despite concerns about safety in people over 50 years of age. In any case, in the points of good practice, the experts give specific indications for the management of mucosal patients with special situations.

It is clear that progress has been made to improve treatment and bring it closer to the places where patients usually live, but it is also clear that there are many scientific gaps that force us to accept the concept of experts as points of good practice. The new clinical studies must be methodologically more rigorous so that their results have a better level of evidence. It is also true that in rare, long-standing clinical forms of leishmaniasis in which there is no certainty that cure will be achieved with any of the treatments tried, current and future publications, even if they are not strictly under GCP,

should be reviewed individually and taken into consideration - even as points of good practice - to answer the questions that still persist.

S22. MOLECULAR PATHOLOGY AND STRATIFICATION OF LEISHMANIASIS

S22-01: DISSECTING THE IMMUNE LANDSCAPE OF POST KALA-AZAR DERMAL LEISHMANIASIS

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Delineating mechanisms that promote immunopathology are necessary for improving approaches for disease control. Post Kala-azar Dermal Leishmaniasis (PKDL), a sequel of apparently cured Visceral Leishmaniasis is considered as the strongest contender to be the disease reservoir for visceral leishmaniasis/Kala-azar. PKDL is the only dermal variant of leishmaniasis without an animal model and is conspicuously associated with hypopigmentation. This study aimed to delineate the key immune alterations at the lesional sites of PKDL and define their contribution, if any, in supporting parasite survival and facilitating hypopigmentation. At lesional sites of PKDL, the status and distribution of key immune cells, namely dendritic cells ($CD1a^+$), $CD4^+$ and $CD8^+$ T-lymphocytes, $CD20^+$ B-lymphocytes, $CD68^+$ monocytes/macrophages and $CD66b^+$ neutrophils was evaluated by immunohistochemistry (IHC). Additionally, the status of melanogenic enzymes, Tyrosinase (TYR), Tyrosinase Related Protein 1 (TRP1) and Microphthalmia-associated Transcription Factor (MITF) were evaluated by droplet digital PCR, along with the inflammasome signaling cascade (NLRP3, Caspase-1, IL-1β) analyzed by RNA-in situ hybridization and IHC and as also the circulating levels of cytokines and chemokines by a Bioplex assay. In comparison to skin from healthy individuals, PKDL cases demonstrated an increased infiltration of anergic/exhausted $CD8^+$ T-cells

(enhanced PD-1 and granzyme/perforin negative), alternatively activated M2 monocytes/macrophages (arginase-1$^+$, CD206$^+$) and activated neutrophils (increased CD64$^+$, myeloperoxidase and neutrophil elastase) along with a decreased presence of dendritic cells. There was a dramatic reduction in *TYR*, *TRP-1* and *MITF* coupled with a strong expression of NLRP3, Caspase-1 and IL-1β, that was mainly within keratinocytes. There was an absence of Ki67 positivity that was accompanied by increased plasma levels of chemokines, along with pro-inflammatory and regulatory cytokines. In PKDL, modulation of the immune landscape conferred susceptibility to intracellular infection, and as it was accompanied by an overarching pro-inflammatory milieu, it translated into an impairment of melanogenesis, collectively suggesting that modulation of the immune environment is a promising therapeutic option.

Keywords ALTERNATIVE ACTIVATION; INFLAMMOSOME; MELANOGENESIS; POST KALA-AZAR DERMAL LEISHMANIASIS (PKDL); VISCERAL LEISHMANIASIS

S22-02: CD8 T CELLS AND HYPOXIA IN CUTANEOUS LEISHMANIASIS

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Cutaneous leishmaniasis is a disease caused by *Leishmania* parasites and exhibits a wide range of clinical manifestations from self-healing lesions to chronic debilitating infections. Currently there are no vaccines for this disease, and the drugs used to resolve the infections are often ineffective. Although the parasites are important determinants of disease severity, the immune response itself causes a large amount of pathology. CD8 T cells have been shown to play a dual role in disease by being both protective when they produce IFN-γ, but pathogenic when they mediate inflammation-inducing cell death in lesions. We found that IFN-γ-producing protective CD8 T cells are restricted to draining lymph nodes, whereas cytotoxic and therefore pathogenic CD8 T cells are found in leishmanial lesions in both experimental murine models of the disease and in patients. Our results suggest that this dichotomy in CD8 T cell function is a response to the tissue microenvironment and that protective IFN-γ-producing CD8 T cells become cytolytic once they enter leishmanial lesions. Transcriptional analysis comparing antigen-experienced CD8 T cells purified from lesions and draining lymph nodes showed that hypoxia is a signature of pathogenic CD8 T cells present in *Leishmania*-infected skin. Transcriptional profiling in patients showed that hypoxia is a signature of human cutaneous leishmaniasis and correlates with *GZMB* gene expression. We were able to demonstrate that leishmanial lesions are hypoxic and that exposure to the hypoxic microenvironment promotes conversion of protective CD8 T cells into cytolytic, GzmB-expressing, CD8 T cells. Deletion of HIF-1a, a master regulator of hypoxia, decreases the expression of GzmB by CD8 T cells within lesions, demonstrating that hypoxia leads to the development of pathogenic CD8 T cells in cutaneous leishmaniasis. Overall, these studies provide

information that will be foundational in understanding the immune responses mediating disease in cutaneous leishmaniasis.

Keywords GZMB GENE; IFN-γ; *Leishmania;* HYPOXIA; SELF-HEALING

S22-03: REGULATION OF INFLAMMATORY RESPONSE IN PATIENTS WITH CUTANEOUS LEISHMANIASIS

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The lesion infiltrate from the ulcerated phase of CL is predominantly composed by lymphocytes, NK cells and macrophages, and few parasites are observed. In this phase of the disease strong inflammatory response, with high levels of TNF and IL-1β, is associated with disease severity. Also, an important role for CD8+ T and NK cells in the pathogenesis of CL has been documented. We have shown that these cells produce high amounts of granzyme B, enhancing pro-inflammatory responses at lesion site. Before the ulcer develops, CL individuals present a large regional lymphadenopathy, followed by the appearance of an exoulcerative papule. Patients in this initial phase of the disease have higher parasite load and higher rate of therapeutic failure when compared to ulcerated CL individuals. To investigate the immune response at lesion site in the initial phase of the disease, we cultured lesion fragments from CL patients in the initial and ulcerated phase of the disease. We observed that CL patients in the initial phase of the disease produce high levels of angiogenesis-related factors, NK cells activation factors (IL-2 and IL-15) proinflammatory cytokines (TNF and IL-1β), granzyme B, and T and NK cells recruitment factors (CXCL9 and CXCL10). Addition of exogenous IL-10 to lesions cells from CL patients in the initial phase of the disease was only able to decrease the production of IL-2 and TNF. Signaling through PPAR-gamma, a nuclear lipid receptor, often leads to anti-inflammatory and wound healing responses. Addition of Omega 3, a PPAR-gamma ligand, to *Leishmania* antigen-stimulated PBMC decreased proinflammatory cytokines and

increased parasite killing. Our data suggest that increased production granzyme B, CXCL9 and CXCL10 is associated with ulcer development in CL patients and show that cells infiltrating the lesion site are mostly unresponsive to IL-10. Furthermore, signaling through PPRA-gamma might benefit patients, decreasing inflammatory response and enhancing parasite killing.

S22-04: UNRAVELING THE IMMUNOLOGICAL SIGNATURES OF HEALING RESPONSES TO OPTIMIZE THERAPEUTICS FOR CUTANEOUS LEISHMANIASIS

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Despite clinical resolution of cutaneous leishmaniasis (CL), viable *Leishmania* persist in a high proportion of individuals, indicating that determinants other than parasite clearance are involved in antileishmanial drug efficacy. How then do immunological functions and antimicrobial drug effects interact to drive the clinical outcome of anti-leishmanial therapy? Are immunological functions targetable pharmacodynamic parameters of anti-leishmanial therapy? Our group and others have taken upon the task of characterizing what comprises healing and non-healing outcomes of human CL, towards identifying targets and approaches for optimization of antileishmanial therapies. However, this process is extensive and once immersed in it, seemingly reductionist as the outcomes of particular pieces of the puzzle are unlikely to be generalizable to the spectrum of infection and disease of CL on a global scale. How to efficiently address this complexity? This seminar will address the opportunities and challenges of translational research on CL, with an emphasis on immunopathology as a central aspect for therapeutic interventions.

S23. FUTURE PROSPECTS IN THE TREATMENT OF CUTANEOUS LEISHMANIASIS FORM

S23-01: IS THERE A PLACE FOR IMMUNOMODULATORS IN THE TREATMENT OF CL?

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Localized Cutaneous Leishmaniasis (CL) is the most common form of presentation of Tegumentary Leishmaniasis, accounting for more than 90% of cases in the endemics regions. This form is characterized by a single or few ulcerated lesion(s), with raised and erythematous edges, preferably located in the lower limbs, and less commonly arms, trunk and face. The standard treatment for CL in many countries is performed with pentavalent antimony (Sb^v) at a dose of 15-20mg/kg per day for 20 days, as recommended by the Ministry of Health of Brazil. However, failure to respond to treatment has been described in up to 40% of patients in Bahia, Brazil and the long period of 60 to 90 days required for healing of the ulcerated lesion indicates the need for the use of alternative drugs. Currently, alternatives include other parenteral drugs such as pentamidine (with low efficacy against L. braziliensis) and amphotericin B. However, their use is limited either by toxicity or because, as with Sb^v, the parenteral route challenges adherence and regularity of treatment in rural areas. Additionally, Amphotericin B requires hospitalization, being reserved for the treatment of patients who do not respond to conventional treatments or

with severe forms of CL. In this context, the need to develop more effective treatments is imposed, with the objective of increasing the cure rate, reducing morbidity and absenteeism caused by the disease. The use of multidrug therapy in the treatment of diseases caused by intracellular agents has long been indicated for the treatment of tuberculosis and leprosy. Also with regard to cutaneous leishmaniasis, there are several examples of the use of more than one drug. In CL and mucosal leishmaniasis (ML), tissue damage is more related to the host's immune response than to a cytotoxic action of the parasite. Since there is evidence that T cell activation and the frequency of T cells expressing TNF or IFN are associated with the size of the leishmaniasis ulcer, association with immunomodulatory agents has been used as adjuncts in the treatment of CL and ML. In ML, it is known that the association of Sb^v with pentoxifylline, a TNF inhibitor drug, is more effective than Sb^v in monotherapy. The association also reduces healing time and cures patients who are refractory to the use of Sb^v. In CL, GM-CSF has the property of modulating the immune response and associated with Sb^v both subcutaneously and topically, it is more effective than Sb^v and placebo, also accelerating the healing time. More recently, miltefosine associated with the topical use of a cream containing 0.1% GM-CSF (M+GM group) was compared to conventional treatment with Sb^v (Sb^v group) and to miltefosine with a placebo cream vehicle (M+P group) in 133 patients with CL caused by *Leishmania braziliensis*. The final results showed a cure rate of 76% for the M+GM group, 77% for the M+P and only 44% for the Sb^v group. A shorter healing time was also documented in the two groups that used miltefosine. Although GM-CSF 0.1% in cream did not show an adjuvant effect, miltefosine confirmed its superiority over Sb^v. However, it is worrying that the best cure rate of miltefosine remains below 80%, which reinforces the importance of seeking other alternatives for association. One such alternative is another cytokine, the granulocyte colony-stimulating factor (G-CSF). It is a 19 kDa glycoprotein that stimulates the production of granulocytes by the bone marrow, stimulating also the differentiation and function of neutrophils. In addition, G-CSF also has an important role in promoting skin healing by stimulating the proliferation of keratinocytes, being produced by fibroblasts when interacting with these cells. G-CSF was used experimentally in patients with toxic epidermal necrolysis and in dystrophic epidermolysis bullosa to accelerate the epithelialization and healing processes. Furthermore, G-CSF

induces IL-10-producing regulatory cells, in addition to negatively interfering with the function of CD8+ cytotoxic cells that are recognized as important agents of tissue damage in CL. All these actions of G-CSF may be important in controlling the intense tissue inflammation that implies in the appearance and maintenance of the ulcer, as well as in the stimulation of skin healing. Therefore, it is likely that the association of G-CSF with conventional treatment of CL will have a positive impact, increasing the cure rate and accelerating healing, avoiding the need for new series of treatment. Another molecule with immunomodulatory capacity to be used in inflammatory diseases is the Sm29 protein located on the surface of *Schistosoma mansoni* adult worms. Preliminary data showed that recombinant Sm29 reduced *in vitro* the production of IFN-γ and TNF and induced high levels of IL-10, in mononuclear cells of more than 60% of patients with CL, thus identifying the potential of Sm29 in the regulation of a Th1-type immune response. Both molecules are being tested as topical adjuvants to standard Sb^{v} treatment of CL in Corte de Pedra, Bahia, Brazil.

Keywords G-CSF; GM-CSF; PENTAVALENT ANTIMONY, IFN-γ; *Leishmania braziliensis*

S23-02: BUILDING THE PATHWAY FOR TRANSLATIONAL DRUG DEVELOPMENT OF ORALLY ACTIVE NEW CHEMICAL ENTITIES FOR CUTANEOUS LEISHMANIASIS

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Leishmaniasis is a complex vector-borne disease caused by the protozoan parasite *Leishmania spp.* Infection results in diverse clinical manifestations, ranging from localised skin ulcers in cutaneous leishmaniasis (CL) and potentially fatal systemic disease in visceral leishmaniasis (VL), to complicated post-kala-azar dermal leishmaniasis (PKDL) and muco-cutaneous lesions (MCL). CL remains a neglected disease for which new, safe, and orally efficacious drugs are urgently required. Although progress has been made recently, R&D efforts against CL lag behind, particularly the development of robust animal models and efficient translational tools. DND*i* has been actively engaged in leishmaniasis R&D for over 15 years, and, together with multiple partners, has built an unprecedented portfolio of late-lead series, preclinical and clinical candidates against VL, originating from different chemical classes with different mechanisms of action against *Leishmania* parasites. This pipeline of new chemical entities (NCEs) provides a strong basis for advancing towards oral leishmaniasis treatments and provides options to overcome attrition during development and to identify one or more combination treatments. DNDi's CL programme strategy is to leverage this rich VL portfolio by expanding its development to simultaneously identify new oral CL treatments (and potentially

combinations with immunomodulators). This objective is strongly supported by previously generated data and ongoing investigative efforts, which clearly demonstrate the capacity of these candidates to be "expanded" for CL. Data includes potent *in vitro* profiles against a panel of CL-causing strains, *in vivo* proof-of-concept studies in animal models and skin distribution potential for some of these NCE candidates. Now, DND*i* has partnered with an international group of experts to further explore these NCEs via detailed PBPK studies and PKPD analysis in skin using animal models of the disease. This approach is aimed at filling in translational gaps currently hindering or even precluding the upstream clinical development of CL drugs. This presentation will focus on reviewing previously published data for two NCE candidates and discussing the implementation of the sort of translational platform that could enable CL drug development and could possibly be expanded to other parasitic skin diseases.

Keywords CUTANEOUS LEISHMANIASIS; DRUG DISCOVERY; TRANSLATIONAL RESEARCH; SKIN PHARMACOKINETICS

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S23-03: TOPICAL PAROMOMYCIN IN CUTANEOUS LEISHMANIASIS: SUPPORTIVE CLINICAL DATA AND A REGULATORY ROADMAP

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Cutaneous leishmaniasis is a disfiguring infection of the skin that affects hundreds of thousands of people each year but for which treatment options remain limited. Patients in many parts of the world must often rely on physical interventions or injectable therapies which can be painful, toxic, or difficult to access. Safe, effective, and accessible outpatient therapies remain a top public health priority for the control of this disease. We will present Phase 2 and 3 clinical trials data and a potential FDA regulatory pathway for ATI-1801, a novel topical formulation of antiparasitic agent paromomycin (15% w/w) for the treatment of uncomplicated cutaneous leishmaniasis. The safety and effectiveness of ATI-1801, administered once daily for 20 days, was evaluated in two Phase 2 and two Phase 3 studies. ATI-1801 was well tolerated in both children and adults across all studies, with no treatment related Grade 3+ adverse events reported, and most mild-to-moderate adverse events localized to the treatment site. Efficacy was also demonstrated in an initial Phase 3 study completed in Tunisia. The randomized, vehicle-controlled study met its primary endpoint, with a significant improvement in the rate of index lesion clinical cure reported for patients receiving ATI-1801 (81.6%; n = 102/125) compared to control (58.4%; n = 73/125; p-value < 0.001). A similar cure rate for patients receiving ATI-1801 was reported in a subsequent Phase 3 study completed in Panama (77.8%; n = 154/198) but no vehicle control was included. Available clinical data suggests ATI-1801 may represent a safe and effective treatment option for patients with uncomplicated cutaneous leishmaniasis and supports continued development of ATI-1801 to support licensure.

S23-04: UNMET NEEDS IN CUTANEOUS LEISHMANIASIS DIAGNOSIS AND 2030 ROAD MAP TARGETS

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Globally, cutaneous leishmaniasis (CL) is on the rise with high burden foci in Latin America, northern Africa and the Middle East. The different forms of CL affect up to 1 million people worldwide and are associated with disability, stigmatization and mental health problems, causing about 260,000 DALYs in recent years. Early diagnosis of CL is one of the core interventions for reducing the burden of disease. This is usually based on a combination of clinical-epidemiological diagnosis and laboratory tests (mainly microscopy). The latter is not always possible, meaning that in some countries an important number CL cases are put on (toxic) treatment without laboratory confirmation. The main target set for CL in the Road Map for Neglected Tropical Diseases (NTDs) is that by 2030 at least 85% of all CL cases are detected and reported and 95% of these are treated. Currently, the number of countries where this is actually happening is unknown, and this is partly due to the lack of adequate diagnostics. Despite its low and variable sensitivity, microscopy is still the reference method for diagnosing CL. Besides, the equipment and trained personnel required for it are not always in place. Immunological tests are of limited use as they basically inform of exposure to *Leishmania*. Nucleic acid amplification tests have a very good diagnostic performance and are advantageous in long duration lesions, but barriers to implementation have not been addressed yet and their use is limited to reference centres. A rapid diagnostic test (RDT) for leishmanial antigen detection would be ideal, but the only one that is available has limited sensitivity and variable across regions and *Leishmania* species. Better diagnostics are needed to reach the 2030 targets highlighted by WHO in the Road Map for NTDs, being a critical action to improve the affordability and sensitivity of RDTs for detection of cases. The WHO Diagnostic Technical

Advisory Group (WHO DTAG) was established as the principal advisory group to WHO on NTD diagnostics in order to ensure a unified approach is used to identify and prioritize diagnostic needs and to inform WHO strategies and guidance on the subject. One of the priority diagnostic needs identified by the WHO DTAG was an RDT for confirmation of suspected cases of cutaneous leishmaniasis at peripheral health facilities. Point-of-care tests for early diagnosis of CL benefit both patients and communities by early identification of those who need treatment, thereby reducing the risk of both sequelae and ongoing *Leishmania* transmission. It is therefore important that new tests be developed to meet the needs of the target population and the requirements for implementation in resource-limited settings, where most cases of cutaneous leishmaniasis occur. A target product profile for a test addressing these needs was developed by FIND in collaboration with DNDi, WHO and CL experts, this was recently reviewed, updated and endorsed by the WHO DTAG. New diagnostic options that are affordable, simple, specific, sensitive, and robust are urgently needed to advance CL control. CL mainly affects poor populations that have limited resources to access health care. The resources devoted so far to improve CL management and control are scarce, but interventions that allow early detection and treatment would contribute to reducing a huge burden of disease, which is often overlooked, and indirectly, poverty.

Keywords NEGELCTED TROPICAL DISEASES; *Leishmania*; TREATMENT

S24. LEISHMANIASIS AND MOVEMENT: IMPORTED LEISHMANIASIS BY TRAVELERS AND MIGRANTS

S24-01: DIAGNOSIS, THE IMPORTANCE OF TYPING

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Leishmaniasis comprises a group of infectious diseases with a global distribution, all of them caused by parasites of the genus *Leishmania* and transmitted by sandflies. Leishmaniasis is included in the group of neglected tropical diseases and shows a wide variety of clinical presentations, ranging from self-healing cutaneous forms to more severe visceral forms, which can be fatal in the absence of treatment. The ecological and epidemiological complexity of transmission, with different pathogenic species of parasites, different species of sand fly vectors and domestic and wild animal reservoirs, constitute a major challenge for disease control. The geographical extension and incidence of leishmaniasis in endemic areas are increasing as a result of factors such as climate change, environmental transformations, population movements or the health conditions of the population, all of which contribute to change the current distribution of the eco-epidemiological cycles of leishmaniasis and to increase the complexity of the transmission dynamics of the parasite. Situations such as armed conflicts and refugee movements, and international travel and tourism have led to an increase in the number of imported leishmaniasis cases. This occurs both in non-endemic areas and in areas where leishmaniasis is already endemic but is caused by another species of the parasite. A good example of this is Spain, where autochthonous cases of visceral (VL) and

cutaneous (CL) leishmaniasis caused by *L. infantum* are reported, but where numerous cases of CL are also diagnosed in travelers from Latin America, the Middle East or North Africa. In these cases, due to the great variability of *Leishmania* spp, makes parasitic characterization essential to establish the appropriate treatment. Molecular characterization of parasites is a rutinary activity in our lab, which is the National Reference Laboratory for Human Leishmaniasis as well as WHO Collaborating Centre for Leishmaniasis since 1997. We have been working for years on the diagnosis and identification of *Leishmania* species from imported cases of leishmaniasis that arrives in our country from endemic areas of Latin America, North Africa or the Middle East, in order to help clinicians to establish the most appropriate treatment. In addition, we have actively collaborated with the identification of the *Leishmania* species that cause cutaneous and visceral leishmaniases in different African countries such as Chad, Cameroon, Ivory Coast or Ghana, which can certainly help to understand the epidemiology of leishmaniasis in West Africa. In addition, the lab also carries out phylogenetic and molecular epidemiology studies on leishmaniasis, that allows detecting the possible introduction of new species of *Leishmania* and the appearance of "hybrids". In Mediterranean endemic countries such as Spain, Italy or Greece, with an already established transmission cycle for *L. infantum*, the massive arrival of immigrants from the Middle East and North Africa, where L. tropica and L. major are also present, generates a risk of introduction of these species in southern Europe and the appearance of "hybrids" that must be evaluated. The molecular characterization of the parasites can be carried out through the amplification and genetic sequencing of specific markers, molecular typing by MLMT and more recently through the genetic sequencing of the maxicircles of the kDNA of the parasites. These molecular approaches allow not only to identify the species of *Leishmania* but also to find genotypic variants within the same species, which apart from the epidemiological interest may have relevance in the clinic and treatment of the disease. We are also aware of the importance that the export of leishmaniasis cases originating in Mediterranean countries can have at a European level and that affect tourists from northern Europe who visit them every year. In these cases, it is necessary to carry out the genetic characterization of the parasites that cause these cases of "exported" leishmaniasis, in order to include them in our phylogenetic and surveillance studies.

S24-02: CLINICAL DIVERSITY OF IMPORTED LEISHMANIASIS IN NORTH AMERICA

Naomi Aronson MD

Uniformed Services University, Bethesda MD USA

Restricting this discussion to otherwise healthy adults, in this talk various clinical presentations of leishmaniasis acquired abroad (outside of North America) and imported into US/ Canada will be shared. This will include available diagnostic testing results and clinical presentation of Old and New World cutaneous infection, New World mucosal leishmaniasis and asymptomatic visceral leishmaniasis. This talk will highlight the diversity of these presentations and consideration for species identification to guide optimized therapy.

S24-03: TARGETED TREATMENT

Rogelio López-Vélez

WHO Collaborating Centre for Clinical Management of Leishmaniasis. Ramón y Cajal University Hospital-IRYCIS. Madrid, Spain.

What is the best treatment option for a disease with different clinical forms, produced by different parasites, acquired in different geographical areas of the world in different hosts?

What is the clinical form of leishmaniasis?
-Visceral leishmaniasis (VL)
-Tegumentary leishmaniasis: cutaneous leishmaniasis (CL): simplex or complex, mucosal leishmaniasis (ML), diffuse cutaneous leishmaniasis, disseminated cutaneous leishmaniasis, leishmaniasis recidivans, post kala-azar dermal leishmaniasis (PKDL).

Type of patient: Special host?
-Immunosuppressed: HIV, anti-TNF alpha, transplant, other
-With co-morbidity/underlying disease: kidney disease, liver disease, tuberculosis, malnutrition, other
-Elderly, infancy
-Pregnant woman
-Relapsed/failure case

Place of acquisition / *Leishmania* sp. Involved
-The same drug and dosing regimen may not have the same efficacy depending on the peographical area of use. In Africa, the current drugs showed lower rates of efficacy than in Asia
-Increased risk mucosal leishmaniasis (*L. (V.) braziliensis*, *L. (V.) guyanensis*, *L. (V.) panamensis*) areas: South of the Amazon in Peru, Brazil and Bolivia Moderate risk areas are south of Nicaragua to Amazon
-Mediterranean basin (*L. infantum*) for immunocompromised

Treatment options for Tegumentary Leishmaniasis (TL): Treatment for leishmaniasis may be local, systemic or both.

-Physical treatment: thermotherapy, co2 laser, cryotherapy, photodynamic therapy
-Topical drugs: topical paromomycin, topical l-amb, intralesional pentavalent antimonials, intralesional pentamidine isethionate
-Oral treatment: azole drugs, miltefosine
-Im/iv treatment: pentavalent antimonials, liposomal amphotericin b, ab deoxycholate, paromomycin, pentamidine isethionate
-Other treatment: pentoxifylline oral, interferon-gamma (IFN-γ), others

Select the treatment:

-Indications for local treatment (or no treatment): Simple cutaneous leishmaniasis
-Indications for systemic treatment (oral or parenteral): Complex cutaneous leishmaniasis

VL in South East Asia by *Leishmania donovani*

L-AmB (iv) 10mg/kg i.v. as a single dose or 3-5 mg/Kg/daily for 3-5 doses, up to 15 mg/Kg total dose

Alternative: L-AmB 5 mg/kg single dose + PM 15 mg [11 mg base]/Kg/d for 10 d or L-AmB 5 mg/kg single dose + MF >50 kg max 150 mg/d for 7 d or PM 15 mg [11 mg base]/kg/d for 10 days + MF >50 kg max 150 mg/d for 10 d

VL in South East Africa (including Yemen) by *Leishmania donovani*

L-AmB 3–5 mg/kg/d for 6–10 days up to 30 mg/kg total dose or MA 20 mg/kg per day for 17 days + PM 15 mg [11 mg base]/kg/d for 17 days

Alternative: MA 20 mg Sb5+/kg/d for 21 days or PM 15 mg [11 mg base]/kg/d for 14 days + MF allometric for 14 d

VL in The Mediterranean basin and in Brazil by *Leishmania infantum*

L-AmB (iv) 3-5 mg/Kg/daily for 5-10 doses, up to 21-30 mg/Kg total dose

Alternative:MA 20 mg Sb5+/Kg/d for 28 d or MF 50 mg tid for 28 days

HIV-VL coinfection and VL in the immunocompromised patient
L-AmB (iv) 3-5 mg/Kg/daily for 8-10 doses (days 1–5,10,17,24,31,38) up to ≥40 mg/Kg total dose (*)

HIV-VL coinfection South East Asia
L-AmB (iv) 5 mg/Kg/daily for 6 doses (days 1, 3, 5, 7, 9,11) up to 30 mg/Kg total dose + MF 50 mg bid for 14 days (*)

HIV-VL coinfection East Africa
L-AmB (iv) 5 mg/Kg/daily for 6 doses (days 1, 3, 5, 7, 9,11) up to 30 mg/Kg total dose + MF 50 mg bid for 28 days (*)

(*) Antiretroviral therapy should be initiated. Secondary prophylaxis should be given till the CD4 count is > 200/µl: L-AmB 3-5 mg/Kg or MA 20 mg Sb5+/Kg/d or Pentamidine 4 mg/kg/d, every 2-4 weeks.

Follow up:

Tegumentary leishmaniasis: Cure outcomes as defined by the World Health Organization: "initial response" lesion resolution 6-9 weeks (42-63 days) after final treatment; "initial cure" lesion resolution after 90 days follow up; "definitive cure" lesion resolution after 180-360 days of follow up. Repeat parasitological testing is not recommended if appears to be healing

Visceral leishmaniasis: Therapeutic failure or relapse is defined by the persistence or return of symptoms and confirmation of the persistence of the parasite after treatment. Failure generally occurs in the first month and recurrence at 6–12 months. The cure rate for immunocompetent patients is above 98% and resistance to these drugs is rare (relapses can occur up to 12 months after treatment). There is a good correlation between clinical progression and parasitological cure, thus there is no need to perform test of cure after treatment. Clinical follow-up at 6 and 12 months after treatment

S25. BIOMARKERS FOR DIAGNOSIS OF LEISHMANIASIS

S25-01: BIOMARKERS IN VISCERAL LEISHMANIASIS PATIENTS COINFECTED WITH HIV

Wim Adriaensen

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Compared to cure rates of around 90-95% in immunocompetent VL patients, treatment of patients with a concurrent Human Immunodeficiency Virus-1 (HIV) infection (referred to herein as 'VL-HIV patients') frequently fails (cure rate <50%). VL-HIV coinfection is common in endemic regions of East-Africa, Brazil and India. Treatment failure results in extended treatments and case-fatality rates up to 25%. This is particularly true for East Africa where antileishmanial drugs show lower efficacy rates and HIV prevalence rates of 10-20% are reported among VL patients. Despite a century of research to identify low-invasive test-of-cure assays or predictive biomarkers to assess treatment efficacy in VL patients, organ aspiration for parasite enumeration remains the sole approach to date. This invasive and high risk procedure limits treatment monitoring, leading to unreliable clinical assessment of cure and high VL relapse rates, in particular among HIV coinfected patients. The lack of an alternative test-of-cure also poses a major obstacle in clinical trials and the development of new treatments. Hence, the development of a less-invasive alternative to assess treatment efficacy represents an urgent and important unmet clinical need, as has been prioritized in the WHO NTD roadmap. HIV patients in particular due to their routine ART follow-up also form a relevant target group for a possible screen-and-treat strategy in endemic areas, requiring biomarkers of VL

development. In this talk an overview of promising alternative biomarkers for diagnosis and treatment monitoring of VL-HIV patients will be discussed. Most alternative strategies currently being explored are based on the decline in parasite load detected via molecular assays or derived antigens with antibody- or antigen-based tests. Limitations and advantages of each assay will be discussed in immunocompromised individuals. Moreover, the recent progress in biomarkers reflecting the host's immunological recovery, including whole blood cytokine-release assays will be presented. In addition, progress in promising avenues, like the development of minimalistic blood-based transcriptomic signatures by combining machine learning algorithms will be shown. Here, we identified a 4-gene pre-post signature (*PRSS33, IL10, SLFN14, HRH4*) that reflected relevant pathways towards cure and could accurately discriminate treatment outcome at end of treatment (Day 29) in a heterogenous group of VL-HIV patients with an average area-under-the-ROC-curve of 0.95 (CI: 0.75–1.00).

Keywords PRSS33; IL10; SLFN14; HRH4; VL-HIV

S25-02: BIOMARKERS IN PKDL

Eduard E. Zijlstra

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Post-kala-azar dermal leishmaniasis (PKDL) follows visceral leishmaniasis (VL; kala-azar) caused by *L. donovani* in up to 60% of cases. It is characterized by an asymptomatic skin rash consisting of macules, papules, or nodules or may have a more polymorphic appearance. Appropriate treatment is currently being investigated in several studies. The response to treatment is difficult to assess as healing may be slow and parasitological and immunological cure may precede clinical cure. Biomarkers are therefore needed; these may be clinical, parasitological, serological, immunological, pathological, or combinations. In this presentation, a review will be given on current biomarkers and promising developments will be discussed.

S25-03: DIAGNOSIS OF VISCERAL LEISHMANIASIS IN AN ELIMINATION SETTING: A VALIDATION STUDY OF THE DIAGNOSTIC ALGORITHM IN INDIA

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Visceral leishmaniasis (VL) is on the verge of elimination on the Indian subcontinent. Nonetheless, the current low VL-incidence setting brings along new challenges, one of which is the validity of the diagnostic algorithm, based on a combination of suggestive clinical symptoms in combination with a positive rK39 Rapid Diagnostic Test (RDT). With this study, we aimed to assess the positive predictive value of the diagnostic algorithm in the current low-endemic setting in India, by re-assessing the newly diagnosed VL patients with qPCR on venous blood as the reference test. In addition, we evaluated the specificity of the rK39 RDT, by testing apparently healthy individuals (without clinical VL) with the rK39 RDT. Participants were recruited in Bihar and Uttar Pradesh, India. VL patients diagnosed based on the diagnostic algorithm were recruited through six Primary Health care Centers (PHCs); non-VL cases were identified through a door-to-door survey in currently endemic, previously endemic, and non-endemic clusters, and tested with rK39 RDT, as well as, if positive, with qPCR on peripheral blood. We found that 95% (70/74; 95% CI 87-99%) of incident VL cases diagnosed at the PHC level using the current diagnostic algorithm were confirmed by qPCR. Among 15 424 apparently healthy

(without clinical VL) individuals, 39 were rK39 RDT positive, reflecting a specificity of the test of 99.7% (95% CI 99.7 – 99.8%). The current diagnostic algorithm combining suggestive clinical features with a positive rK39 RDT still seems valid in the current low endemic setting in India.

Keywords VISCERAL LEISHMANIASIS; DIAGNOSTIC ALGORITHM; rK39 RAPID DIAGNOSTIC TEST; qPCR

Financing Supported by SPEAK India Consortium by a grant from Bill & Melinda Gates Foundation

S26. CELL BIOLOGY AND *Leishmania* INFECTION

S26-01: THE INTERPLAY BETWEEN TOLL-LIKE RECEPTORS AND SERINE PEPTIDASES IN THE *Leihmania donovani* INFECTION

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Mammalian serine peptidases of the S1A family (trypsin family) are involved in many key physiological processes such as blood coagulation and complement activation and are present in the granules of neutrophils and of mast cells. We have identified in the genome of *Leishmania sp.*, genes encoding inhibitors of trypsin-family serine peptidases (ISP), homologues of bacterial ecotins. In *L. major*, ISP2 inhibits neutrophil elastase (NE)-dependent activation of TLR4 in macrophages and prevents the subsequent microbicidal responses, being required for sustained infection *in vitro* and in the mouse model. In contrast, *L. donovani* does not express detectable ISP2 and the peptidase activity of NE was found to be required to sustain parasite intracellular growth in macrophages through the induction of IFN-® via TLRs. We will discuss the molecular pathways associated with IFNb induction upon infection with *L. donovani* promastigotes. We found that both TLR4 and TLR2 act independently to induce gene expression of Type-I interferons (IFN-I), OASL2, SOD1, and IL10 by infected macrophages. NE activity is crucial for this molecular network and the overexpression ISP2 in *L. donovani* prevented induction of (IFN-I) upon infection of macrophages. NE acts at multiple points during infection, affecting the kinetics of acquisition of endosomal markers in parasite-containing phagosomes and enhancing IRF3 levels in the nucleus of the infected macrophage.

Furthermore, we found that the cytoplasmic double-stranded RNA sensor, protein kinase R (PKR), is required for the induction of IFN-I and for parasite intracellular growth. In parallel, TLR3, an endosomal RNA sensor, is also required for IFN-I expression and for parasite sustained intracellular burden. These findings highlight how *L. donovani* promastigotes engage innate responses in infected macrophages via TLR2, TLR4, and TLR3, via downstream PKR, to induce the expression of pro-survival genes in the host cell, and guarantee parasite intracellular growth.

Keywords VISCERAL LEISHMANIASIS; SERINE PEPTIDASES; INTERFERON; TOLL

S26-02: MACROPHAGE MITOCHONDRIAL BIOGENESIS AND METABOLIC REPROGRAMMING INDUCED BY *Leishmania donovani* REQUIRED LIPOPHOSPHOGLYCAN AND TYPE I INTERFERON SIGNALLING

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To colonize macrophages, *Leishmania* metacyclic promastigotes employ a panoply of virulence factors, including lipophosphoglycan (LPG), which impairs different host cell processes and rewire host cell metabolism creating a metabolically-adapted microenvironment required for pathogen replication. Whereas previous studies revealed that *Leishmania* alters signaling axes that regulate mitochondrial function, scarce attention has been paid to the characterization of host cell mitochondrial metabolism during *Leishmania* infection and to the effectors involved therein. Here we investigated the mechanisms governing the modulation of macrophage mitochondrial properties by the vacuolar pathogen *Leishmania*. We obtained evidence that induction of mitochondrial biogenesis by *L. donovani* requires the virulence glycolipid lipophosphoglycan, which mediates the expression of key transcriptional regulators and structural genes associated with the electron transport chain. *Leishmania*-induced mitochondriogenesis also requires a lipophosphoglycan-independent pathway involving type I IFN receptor signalling. Stimulation of oxidative phosphorylation by *L. donovani* is also dependent on lipophosphoglycan and supported by glycolysis and the electron transport chain, but in contrast to mitochondrial biogenesis does not require type I IFN signalling. The observation that pharmacological induction of mitochondrial biogenesis enables an avirulent lipophosphoglycan-defective *L. donovani* mutant to survive and replicate in macrophages supports the notion that mitochondrial biogenesis contributes to the creation of a metabolically-adapted environment propitious to the replication of the parasite. This study provides novel insight into the

complex mechanism by which *Leishmania* metacyclic promastigotes alter host cell mitochondrial biogenesis and metabolism during the colonization process.

Keywords *Leishmania*; LIPOPHOSPHOGLYCAN; MITOCHONDRIAL BIOGENESIS; METABOLISM AND METABOLIC FLUX

Financing Supported by the Canadian Institutes of Health Research

S26-03: RNASEQ ANALYSIS REVEALED IN BONE MARROW CELLS OF SUSCEPTIBLE DOGS NATURALLY INFECTED WITH *Leishmania infantum* MODULATION IN CELL MIGRATION AND DNA DOUBLE-BREAK REPAIR PATHWAYS

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Canine visceral leishmaniasis (CVL) can present as a severe debilitating or subclinical form of the disease; progression is dependent on several factors, including the host immunological status. We performed a previous cohort study in an endemic area of visceral leishmaniasis (VL) that identified and classified dogs as resistant or susceptible to CVL. The bone marrow (BM) is one of the tissues of preference for *Leishmania infantum* infection and myeloid cells are the main host cells of the parasite. However, little is known about the impact of immune cellular alterations in the BM on disease severity. Recently, it was demonstrated in a murine model that *L. donovani* infection induces the expansion of hematopoietic stem cells that reside in the BM of infected mice. In the present study, we focused on the response characterization of BM cells from susceptible dogs naturally infected with *L. infantum*. Longitudinal analysis of the hematological profile and peripheral blood counts revealed significantly decreased red blood cell counts,

hemoglobin and hematocrit levels. Reassessment and characterization of BM cellular profile compared to uninfected dogs as controls revealed erythroid cell hypoplasia in susceptible animals. In the characterization by flow cytometry, the T lymphocyte count was significantly higher in the BM of susceptible dogs, when compared to the bone marrow of control dogs. For further BM cell characterization, gene expression profiling by RNA sequencing was performed. Transcriptomic analysis identified 327 differentially expressed genes (DEGs) in susceptible animals versus controls. Enrichment analysis revealed that pathways related to DNA repair were negatively regulated, while pathways related to cell migration were positively and negatively modulated in susceptible dogs. A machine learning algorithm identified a set of four genes (*EGR2*, *FOS*, *TINAGL1* and *ADCY9*) with high power to detect susceptible dogs. Currently, these DEGs and their associated pathways are undergoing validation by RT-qPCR, immunofluorescence and functional assays, such as neutral comet and transwell cell migration assays. To perform validation, new BM samples were collected. RT-qPCR will be used to validate the set of four DEGs identified by bioinformatic analysis and those involved in DNA double-break repair and cell migration pathways. Assays evaluating the migratory behavior of cell subsets in susceptible and healthy dogs were conducted; at first, in a two-dimensional environment, no significant differences were observed in migration profiles between the groups. In addition, assays using a 3D environment will be performed to characterize the mesenchymal migration performed by cells that migrate from the BM to the periphery. Immunofluorescence was also performed to validate proteins involved in cell migration processes and revealed higher RAC expression in BM cells from susceptible dogs when compared to cells from dogs not infected with *L. infantum*. Neutral comet assays will be later performed to identify the double breakage of DNA in the BM cells. In conclusion, the deep transcriptomic analysis of the cells from the BM, one of the central tissues of VL infection, showed profound alterations in the gene expression profile of susceptible dogs to *L. infantum* infection that in the end would explain the fatal outcome of VL in susceptible animals.

S27. *Leishmania* EXTRACELLULAR VESICLES: IMPACT ON DISEASE PROGRESSION

S27-01: EXTRACELLULAR VESICLES FROM NEW WORLD SPECIES OF *Leishmania*: ROLE DURING INTERACTION WITH MURINE MACROPHAGES AND HUMAN PBMCS

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Leishmania infection causes considerable human morbidity and may develop into a deadly visceral form in endemic regions. The parasite infects macrophages where they can replicate intracellularly. Furthermore, they modulate host immune responses by using virulence factors (lipophosphoglycan, glycoprotein-63 and others) that promote survival inside the cells. Extracellular vesicles (EVs) released by parasites are important for cell-cell communication in the proinflammatory milieu modulating the establishment of infection. However, information on the ability of EVs from different *Leishmania* species to modulate inflammatory responses is scarce, especially from those species causing different clinical manifestations (visceral versus cutaneous). The purpose of this study was

to expose murine macrophages and human peripheral blood mononuclear cells (PBMCs) to EVs from three *Leishmania* species from New World including *L. infantum*, *L. braziliensis* and *L. amazonensis*. Briefly, EVs were released from promastigote forms, purified by ultracentrifugation and quantitated by Nanoparticle Tracking Analysis (NTA) prior to murine macrophage exposure. EVs were also checked by Scanning Electron Microscopy (SEM). We used two types of cells including murine macrophages and PBMCs from human donors. For the murine peritoneal macrophage assays, we quantitated NO and cytokine production (TNF-⟨, IL-6 and IL-10) using CBA flex kit (BD Biociences). For PBMCs, we determined IL-32 production using ELISA. For NF-KB translocation, we performed assays in THP-1 cells and the fluorescence was detected using p65 antibody. NTA analysis did not show any differences in the EV sizes among the strains. EVs from *L. braziliensis* and *L. infantum* failed to induce a pro-inflammatory response. EVs from both *L. infantum* WT and LPG-deficient mutant (LPG-KO) did not show any differences in their interaction with murine macrophages, suggesting that LPG solely was not determinant for activation. However, in PBMCs, WT *L. infantum/L. amazonensis* EVs induced higher levels of IL-32 compared to (LPG-KO). On the other hand, in murine macrophages, EVs from *L. amazonensis* were immunomodulatory inducing NO, TNF-α, IL-6 and IL-10 via TLR4 and TLR2. To determine whether such activation was related to NF-┤ B p65 translocation, THP-1 macrophage cells were exposed to EVs. In the same way, only EVs from *L. amazonensis* exhibited a highly percentage of cells positive for NF-┤ B. Our results suggest an important role of EVs in determining the pattern of immune response depending on the parasite species and the host. For *L. infantum*, LPG deficient-EVs were not determinant for the activation of murine macrophages. However, this activation was very important to induce IL-32 in PBMCs. Depending on the host and *Leishmania* species, EVs may trigger a diverse immune activation. Those polymorphisms during this process may be determinant for the wide spectrum of the clinical manifestations that they cause.

Keywords EXTRACELLULAR VESICLES; *Leishmania*; HOST-PARASITE INTERACTION; INNATE IMMUNITY; LIPOPHOSPHOGLYCAN (LPG), IL-32

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S27-02: EV-DRIVEN GENETIC EXCHANGE AS A NOVEL MECHANISM OF DRUG RESISTANCE IN EUKARYOTIC PARASITES

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Extracellular vesicles (EVs) are nano-sized vesicles secreted by all eukaryotic cells whose contents (proteins, DNA/RNAs, lipids) vary as a function of their cellular origins. EVs have been the focus of numerous studies due to their involvement in intercellular communication. However, the potential roles of EVs in the survival and spread of drug-resistant parasites remain unexplored. Considering that the molecular content of eukaryotic EVs is a fingerprint of the origin cell, reflecting its physiological/functional status, our team recently explored the composition of leishmanial EVs in the context of drug resistance. In this way, we were able to identify *Leishmania infantum* EVs' core proteome, as well as the proteins specifically enriched in EVs released by antimony-, miltefosine- and amphotericin-resistant parasites. We demonstrated for the first time that drug-resistance mechanisms can induce changes in the morphology, size, and distribution of EVs in *Leishmania*, with drug-resistant parasites releasing larger vesicles in comparison to their wild-type counterparts. Of note, several virulence factors, transcription factors, as well as proteins encoded by drug-resistance genes were identified among the drug-specific enriched proteins. Based on these exciting findings, we then explored the potential transfer of different traits of drug resistance from drug-resistant to naïve parasites. However, horizontal gene transfer (HGT) events had never before been demonstrated in eukaryotic parasites. Herein, we explore the DNA content of EVs derived from drug-resistant parasites, as well as their role in both intra- and interspecies HGT events. Next-generation sequencing and PCR assays confirmed the enrichment of circular amplicons carrying drug-resistance genes associated with EVs. Transfer assays of drug-

resistant EVs showed an import shift in the drug-sensitivity profile of recipient parasites; this phenomenon was confirmed to be induced by the expression of genes transferred by EVs. Moreover, recipient parasites displayed enhanced growth and better control of reactive oxygen species. Overall, our work provides the first evidence that *Leishmania* EVs constitute an efficient platform for HGT, facilitating the rapid transmission of drug-resistance genes while increasing the global fitness of recipient parasites.

Keywords *Leishmania infantum*; EXTRACELLULAR VESICLES; DRUG RESISTANCE; GENOMICS; PROTEOMICS; GENETIC EXCHANGE

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S27-03: *Leishmania* EXOSOMES AND ITS VECTOR: IMPACT ON CUTANEOUS LEISHMANIASIS

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Leishmaniasis, a complex pattern of diseases caused by sand fly-transmitted *Leishmania* sp. causes over 2 million new infections and 30,000 deaths each year. In mammals, *Leishmania* parasites establish a persistent infection by inducing MØ dysfunction through direct manipulation of MØ signaling. We have deciphered the mechanisms whereby *Leishmania* exploits MØ signaling pathways to block microbicidal functions and innate inflammatory responses during infection. Work from my lab discovered that *Leishmania major* GP63 was enriched in *Leishmania* exosomes and to play a pivotal role in those deactivation process of MØ responses. We reported that *Leishmania* exosomes are released in the gut of its sand fly vector and co-inoculated with *Leishmania* promastigotes during blood meals. Co-egested *Leishmania* exosomes were found to exacerbate cutaneous leishmaniasis skin lesions by overproducing inflammatory cytokines fueling Th17 immune response. Recently, *Leishmania* RNA virus 1 (LRV1) infecting certain *Leishmania* species was found to be associated with aggressive mucocutaneous disease triggered in response to this dsRNA virus. However, it was unclear how LRV1 is exposed to the mammalian host cells. In higher eukaryotes, some viruses are known to utilize the host exosome pathway for their formation and cell-to-cell spread. As a result, exosomes derived from infected cells contain viral material or particles. Recently, we found that LRV1 exploits the *Leishmania* exosome pathway to reach the extracellular environment. Biochemical and electron microscopy analyses of exosomes derived from LRV1-infected *Leishmania* revealed that most dsRNA LRV1 co-fractionated with exosomes, and that a portion of viral particles was surrounded by these vesicles. Transfer assays of LRV1-containing exosome preparations showed that a significant number of parasites were rapidly

and transiently infected by LRV1. Remarkably, these freshly infected parasites generated more severe lesions in mice than non-infected ones. Moreover, mice co-infected with parasites and LRV1-containing exosomes also developed a more severe disease. Overall, this work provided evidence that *Leishmania* exosomes function as viral envelopes, thereby facilitating LRV1 transmission and increasing infectivity in the mammalian host.

S28. VECTOR SURVEILLANCE AND CONTROL FOR VISCERAL LEISHMANIASIS ELIMINATION

S28-01: INSIGHTS INTO SUCCESSFUL IRS OPERATIONS IN BIHAR AND JHARKHAND: 2012-2022

Bikas Sinha

CARE India

Indoor Residual Spray (IRS) has been conventionally considered the cornerstone strategy for VL elimination in India. The strategy involves spraying insecticide (currently, alphacypermethrin, a synthetic pyrethroid) indoors using a specified protocol to cover all homes in affected villages twice a year for a period of three years after the last reported case. A large proportion of the VL elimination program budget is dedicated to IRS. This includes the cost of equipment and ancillaries, insecticide procurement and transport, spray squad wages and other incidental costs, besides vast amount of administrative and supervisory time and effort. These costs are prorated to the massive scale of the operations, which, despite substantial declines in incidence, for instance, covers an estimated population of 10.6 million in 35,208 villages in Bihar state this year, engaging 913 spray squads for periods of 45-60 days for each of the two annual rounds. Ensuring effective coverage at this scale is a monumental task, and its accomplishment with the budgets allocated represents a highly efficient operation. CARE India has been closely engaged, since 2012 in Bihar and since 2015 in Jharkhand, in supporting IRS operations end-to-end – targeting, planning, preparing, training, overseeing, evaluating coverage and quality. We present granular analysis and insights from the extensive data and experiences accumulated to deconstruct the journey from an estimated household coverage of IRS of less than 40% a decade ago to a steady 85-90%

across Bihar and Jharkhand. We also present residual questions regarding the role of IRS going forward.

Keywords IRS; VL ELIMINATIONS; BIHAR – INDIA; JHARKHAND – INDIA; VECTOR CONTROL

S28-02: EFFECTIVE SURVEILLANCE LEADING TO EFFECTIVE VECTOR CONTROL POLICY CHANGE - WHAT ARE THE NEXT CHALLENGES

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In 2005, Bangladesh, India and Nepal agreed to eliminate visceral leishmaniasis (kala-azar) as a public health problem. This was to be achieved through improved case detection and treatment, and controlling disease transmission by the vector, *Phlebotomus argentipes*, using indoor residual spraying (IRS). Initially, in 2005 India applied DDT with stirrup pumps for IRS, however, this did not have the desired impact and operational research led to a policy change in 2015; where the pyrethroid alpha-cypermethrin applied with compression pumps was introduced for the IRS programme. To support these changes entomological surveillance was initiated in 2016. Continual surveillance is carried out at eight sentinel sites in the Indian states of Bihar, Jharkhand and West Bengal. IRS coverage is monitored by household survey, quality of insecticide application measured by HPLC, presence and abundance of the vector with xenomonitoring for infectivity using CDC light traps, insecticide resistance is measured with WHO diagnostic assays and case incidence are determined from the VL case register KAMIS.

While COVID19 did impact initially on the IRS control programme and surveillance, with the correct procedures in place both were able to resume quickly. Complete treatment of houses with IRS increased across all sites from 57% in 2016 to rising to >80% in 2022 if partial house IRS coverage is included (except West Bengal). The quality of insecticide application has

improved compared to previous studies, average doses of insecticide on filters papers ranged from 1.52 times the target dose of 25mg/m^2 alpha-cypermethrin in 2019 to 1.67 times in 2018 (due to COVID19 this data is not available for 2020 and 2021). Resistance to DDT has continued to increase, with mortality <40%, but no resistance has been detected to carbamates, organophosphates or pyrethroids. The annual and seasonal abundance of *P. argentipes* declined between 2016 to 2022 with an overall infection rate in 2021 and 2022 of 0.0%. This was associated with a decline in VL incidence for the blocks represented by the sentinel sites from 1.16 per 10,000 population in 2016 to 0.21 per 10,000 in 2021. The effective case detection and management reducing the infection reservoirs for *P. argentipes* in the human population combined with quality IRS keeping *P. argentipes* abundance and infectivity low has reduced VL transmission. Combining effective case management and vector control has now brought India within reach of the VL elimination targets.

Keywords INDOOR RESIDUAL SPRAYING; ELIMINATION; POLICY; SURVEILLANCE

S28-03: NEW TOOLS FOR IMPROVING VECTOR CONTROL

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Insecticide resistance is one of the key challenges faced in vector control globally. Over the past decade, a handful of new public health insecticides have come to market to provide effective alternatives. In the absence of public health insecticide stewardship, vector control is at risk of becoming a redundant strategy for eliminating diseases such as malaria and visceral leishmaniasis. To prolong the longevity of available public health insecticides and to achieve disease elimination targets, quality assurance and monitoring the efficacy of insecticides is crucial. For indoor residual spraying (IRS), the World Health Organization recommends performance monitoring through either cone bioassays or High-Performance Liquid Chromatography (HPLC) analysis of Whatman Grade 1 filter papers. Both methods require specialist skills and equipment, are expensive and do not give timely data for a response. As a result, this aspect of IRS monitoring is often neglected. Electromagnetic (EM) wave sensor technology has been used for real-time monitoring within the food and environmental monitoring industry. In order to address the issues associated with IRS performance monitoring, this technology has been pivoted to detect alpha-cypermethrin, which is used for IRS in the Indian visceral leishmaniasis (VL) elimination programme. A hand-held EM sensor device capable of detecting alpha-cypermethrin within operational settings has been developed to promote and support IRS performance monitoring. Providing a categorical response of whether the insecticide detected is “on target”, “above” or “below”, the sensor device allows rapid spray quality assessment and where appropriate corrective measures to be implemented within a campaign. In addition to routine spray performance monitoring, the device could be used to monitor efficacy and residual decay rates. Final field validation studies for the sensor device were conducted in VL endemic villages within

Muzaffarpur district, Bihar. Training on how to use the device was provided to staff associated to the IRS programme, after which staff were asked to collect readings from houses randomly selected for performance monitoring. Matched readings and filter papers were collected from four IRS walls in 360 houses randomly selected based on different surface (mud, thatch, brick, limewash and cement) type present in the study villages. A total 1440 filter papers were analysed by HPLC and compared to the Alpha-sensor readings. Complimentary user feedback surveys were conducted which included open and closed questions around the user experience of the sensor device and its usefulness within the IRS programme monitoring context. A general inductive approach was used to identify emerging themes in the data and align to study objectives. The application of this electromagnetic sensor will provide immediate measurements of the quality of IRS, allowing for timely corrective action and to ensure an effective dose of insecticide is delivered on to the wall. This will reduce the opportunity for insecticide resistance to develop and promote insecticide stewardship.

S28-04: MODELLING THE CONTRIBUTION OF IRS TO ELIMINATION OF VISCERAL LEISHMANIASIS IN THE INDIAN SUB-CONTINENT

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Because the VL elimination target of <1 case per 10,000 capita at the subdistrict level has been or will soon be met in many areas of the Indian subcontinent (ISC), there is a need to better understand how, when, and where VL transmission may resurge again if and when the target is met and control measures are (partially) suspended. Although it is generally accepted that minimising case detection delay is crucial for (sustained) achievement of the elimination target, the contribution of indoor residual spraying (IRS) has been debated. Entomological surveillance data recently collected on the ISC by the Liverpool School of Tropical Medicine and partners provide a unique opportunity to better understand the potential impact and contribution of IRS towards elimination, as well as to better understand the meaning of particular IRS indicators (e.g., coverage, quality). We therefore conducted a modelling study that evaluates the prospects of VL elimination as a public health problem, potential interruption of transmission, and expected trends when control measures are (partially) suspended after meeting the elimination target. In addition, we quantified the relative contribution of IRS to achievement and sustainability of meeting the elimination target.

S28-05: INTEGRATED ACD IMPLEMENTATION- THE UTTAR PRADESH EXPERIENCE (DASTAK-SRNA)

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PATH, India

Uttar Pradesh the largest state and one of the VL-endemic states in India, has achieved and sustained VL incidence less than one case per 10,000 population annually (the VL elimination target as a public health problem, set by the WHO-SEAR). There is a decline in number of VL cases, however the Post Kala-azar Dermal Leishmaniasis (PKDL) cases have shown an increase in the state. 58 blocks from 14 districts have reported VL/PKDL cases so far. During the post elimination phase, it is important to have a sustainable method for surveillance to continue the Active Case Detection (ACD). Uttar Pradesh state has integrated the active community surveillance with other ongoing government priority program like DASTAK (knocking of the door for fever detection and awareness of Japanese Encephalitis/Acute Encephalitis Syndrome), including leprosy, TB& others.

- Sanchari Rog Niantran Abhiyan (SRNA): Communicable Disease Control Mission
- DASTAK (Knock on the door campaign)

As per the Government guidelines all endemic districts must do at least 3-times ACD in the endemic area. However, Uttar Pradesh is performing ACDs as per the following:

1. Three times during the DASTAK-SRNA in all affected districts/bocks started from 2020
2. Two times during the IRS only in IRS villages
3. Also 2 times by ASHA/partners in selected high-risk villages

State is also planning to integrate this special ACD drive with leprosy and tuberculosis program.

Active Case Detection has now become front line health worker's responsibility also known as Accredited Social Health Activist (ASHA). Partners are engaged in, training, microplanning, and monitoring process. ASHAs who receive training and support during the project duration will remain in the system and continue to provide quality support for sustaining VL elimination activities.

Activities during the DASTAK-SRNA:

- Microplanning and training of ASHA (front line health worker)
- ASHA identifies suspects (fever cases > 2 weeks / skin lesions) & share list with blocks
- Blocks mobilizes suspects to PHC / camp in village / RRT
- Screening of these suspected by Medical Officers
- rK 39 testing for the confirmation of diagnosis
- If found positive, treatment given

In Uttar Pradesh ACD integration started with DASTAK from year 2020. In last 2 years approximately 53% of VL and 90% of PKDL cases have been reported through ACD, of which:

- 25% (7/27) of VL and 30% (14/47) of PKDL have been reported through DASTAK campaign in the year-2021
- 22% (6/27) VL cases and PKDL 13% (6/47) reported through IRS in the year-2021

In year 2021- 41% (27/66) new villages were identified in which 15% (4/27) villages were identified during the DASTAK campaign. So, it is important to integrate ACD component with other ongoing program or campaign, to improve the outreach and sustain Active Case Detection, in the post- elimination settings.

S28-06: INTEGRATED ENTOMOLOGICAL SURVEILLANCE – THE WAY FORWARD

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Entomological surveillance and vector control still remain the key components of the important components of the National Centre for Vector Borne Disease Control (NCVBDC), India to obtain crucial information on vector species, their distribution, density, bionomics, resistance to insecticides efficacy and effectiveness of vector control measures. Since 2016, the NCVBDC has collaborated with the Liverpool School of Tropical Medicine, CARE India and All India Institute of Medical Sciences, Patna with an aim to develop the infrastructure and capacity to operate eight sentinel sites to accelerate the efforts for elimination of Visceral leishmaniasis (VL) in three Indian States namely Bihar, Jharkhand, and West Bengal. These sites were involved in assessment of vector density and determination the impact of indoor residual spraying for policy of the VL elimination, which are essential to validate VL elimination as per WHO criteria. In addition to Visceral leishmaniasis NCVBDC is the nodal agency, under the Ministry of Health and Family Welfare, India for managing five major vector borne diseases, viz.,malaria, lymphatic filariasis, dengue, chikungunya and japanese encephalitis. However, in recent years NCVBDC has been challenged with diminishing entomological man power and infrastructure across the country. Therefore, NCVBDC planned to expand the collaboration to make entomological surveillance to cover all six diseases in an integrated manner India. The proposed integrated entomological surveillance will consist of 18 entomological surveillance sites in Bihar (6), Chhattisgarh (1), Jharkhand (5), Maharashtra (1), Odisha (1), Uttar Pradesh (2), and West Bengal (2). Four villages will be selected in each of the sentinel sites based

on a range of factors such as level of vector borne disease transmission, demography and population at risk. Key entomological indicators that will be collected for each vector will include:

- Species abundance to determine the best time for an intervention and to measure the impact
- Xenomonitoring to determine the transmission of disease in the population and control impact
- Insecticide resistance to ensure interventions are adequate
- Mechanisms of insecticide resistance to assist with planning of alternative interventions
- Ad hoc entomological operational research questions

All entomological data collected along with associated case data and vector control operational data with be incorporated into the bespoke Disease Data Management System (DDMS+ database) established in Bihar, 2013. This will allow for routine reporting to the national programme and stakeholders, allowing for timely analysis of data to substantiate control efforts for informed decision making. Further, data generated from this first integrated entomological surveillance system will be utilized to determine the status of multiple disease transmission, design appropriate vector control and measure the impact of disease control measures in the region.

S29. A GLOBAL VISCERAL LEISHMANIASIS DATA PLATFORM

This symposium will present and discuss the Visceral Leishmaniasis (VL) Data Platform hosted at the Infectious Disease Data Observatory (IDDO). The result of collaborative development between VL researchers, funders, and IDDO, the Data Platform holds 14,628 individual patient data (IPD) from 44 clinical trials assessing the safety and efficacy of antileishmanial treatments between 2000 and 2021. This represents 40% of published IPD since 2000. The data have been curated to provide a standardised resource for the VL research community in order to address persistent knowledge gaps in VL, alongside tools to facilitate standard prospective data collection for future VL clinical trials. Members and observers of the IDDO VL Scientific Advisory Committee will discuss the purpose, use, and potential of data standardisation.

S29-01: KEY CHALLENGES AND OPPORTUNITIES

Mitali Chatterjee

Institute of Postgraduate Medical Education and Research

The conception and development of the IDDO VL Data Platform was an intensely collaborative endeavour with the VL research community. The challenges overcome and the opportunities afforded during platform development highlight the importance of this approach to achieve the goals of the data platform: to collate data and deliver robust science to address knowledge gaps and generate new evidence to improve treatment outcomes.

S29-02: OPTIMISING HISTORICAL DATA AND PLANNING PROSPECTIVELY

Ahmed Mudawi Musa

University of Khartoum

End-to-end solutions are needed across the data lifecycle of clinical studies, with optimisation and standardisation of data of importance for both historical and prospective data collection. Historical data standardisation affords immense opportunity to make the most of research already performed via individual patient data (IPD) meta-analysis. Applying standardisation prospectively optimises data collection for future studies for greater efficiency.

S29-03: DATA PLATFORM POTENTIAL FOR DRUG DEVELOPMENT

Fabiana Alves

Drugs for Neglected Diseases initiative, Seitzerland

IDDO's VL Data Platform has demonstrated its value for drug development. IPD meta-analysis can investigate outstanding questions as to drug efficacy and side effects by overcoming sample size restrictions. Prospective data standardisation through use of Case Report Forms (CRF) in up-coming trials will support even greater harmonisation between trials, increasing the power and impact of results.

S29-04: IMPACT ON POLICY

Saurabh Jain

World Health Organisation

Through its focus on delivering benefits to disease-affected communities, the VL Data Platform holds real potential for policy influence and impact. Translational research in the form of systematic review and IPD meta-analyses can address gaps in global, regional and national knowledge and guidance, and answer issues surrounding risk factors and safety.

S29-05: FUTURE BENEFITS OF THE VL DATA PLATFORM FOR RESEARCH

Philippe Guérin

IDDO

IDDO is actively engaged in several areas of research and resource development. On-going study groups to interrogate the IPD include questions on anaemia and severity risk factors, and an annotated CRF for both uncomplicated VL and VL-HIV co-infection has been developed. Future activities involve further study groups in collaboration with or led by researchers in the VL community, and expansion of the platform to include PKDL data.

S30. IMMUNOPATHOGENESIS AND HOST-DIRECTED THERAPIES IN LEISHMANIASIS

S30-01: THE MAINTENANCE OF *Leishmania*-SPECIFIC CD4⁺ MEMORY T CELLS REQUIRES THE CONTINUOUS PRESENCE OF THEIR COGNATE ANTIGEN

Jude E Uzonna

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Memory T cells play essential role in immunity against viruses, bacteria and protozoan parasites. Although it has been shown that memory CD8⁺ T cells are maintained as a stable pool for extended time periods in the absence of their cognate antigen, whether memory CD4⁺ T cells are sustained in the absence of antigen is controversial. Leishmaniasis is caused by the protozoan parasite belonging to the genus *Leishmania*. Most individuals who recover from cutaneous leishmaniasis acquire a lifelong immunity to reinfection. This infection-induced immunity is believed to be dependent upon the presence of persistent parasites although empirical evidence supporting this is lacking. We found the mice infected with dihydrofoliate reductase thymidylate synthase (dhfr-ts) deficient *L. major*, which are incapable of surviving due to inability to salvage thymidine, lost protection against wild-type *L. major* challenge in 24 weeks, which corresponds to loss of *Leishmania* (PEPCK)-specific CD4⁺ T cells. To test the fate of *Leishmania*-specific memory CD4⁺ T cells in absence of antigen, we generated *Leishmania* PEPCK-specific CD4⁺ TCR transgenic mice (PEG). Using this unique tool, we found that both *in vitro* and *in vivo* generated memory PEG

cells disappeared over time in both MHC II KO or WT mice and this was associated with loss of protection following challenge infections. Similarly, following adoptive transfer of PEG cells and infection with *dhfr-ts L. major* or immunization with PEPCK peptide, PEG cells underwent cell expansion and contraction but completely disappeared in about 300 days. PEG cells also did not persist in mice infected with either *dhfr-ts* or PEPCK deficient *L. major*, but did persist in mice infected with WT parasites. The disappearance of memory PEG cells in either *dhfr-ts* or PEPCK deficient *L. major* infected mice was associated with loss of protection after rechallenge infections with WT *L. major*. Taken together, these results show that the maintenance of antigen-specific memory $CD4^+$ T requires the continuous presence of their cognate antigen.

S30-02: PROTECTIVE CD4+ TH1 CELL-MEDIATED IMMUNITY IS RELIANT UPON EXECUTION OF EFFECTOR FUNCTION PRIOR TO THE ESTABLISHMENT OF THE PATHOGEN NICHE.

Leah S. Hohman[1], Zhirong Mou[2], Matheus B. Carneiro[1], Gabriel Ferland[1], Rachel M. Kratofil[3], Paul Kubes[3], Jude E Uzonna[2], Nathan C. Peters[1]

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Intracellular infection with the parasite *Leishmania major* features a state of concomitant immunity in which CD4+ T helper 1 (Th1) cell-mediated immunity against reinfection coincides with a chronic but sub-clinical primary infection. In this setting, the rapidity of the Th1 response at a secondary site of challenge in the skin represents the best correlate of parasite elimination and has been associated with a reversal in *Leishmania*-mediated modulation of monocytic host cells. Remarkably, while many models of *Leishmania* control imply that Th1 immunity can eliminate parasites following infection and replication within phagocytes, the degree to which Th1 cells are reliant upon the time at which they interact with infected monocytes to mediate their protective effect has not been well defined. In the present work, we report that CXCR3-dependent recruitment of Ly6C+ Th1 effector (Th1EFF) cells is indispensable for concomitant immunity and acute (<4 days post-infection) Th1EFF cell-phagocyte interactions are critical to prevent the establishment of a permissive pathogen niche, as evidenced by altered recruitment, gene expression and functional capacity of innate and adaptive immune cells at the site of secondary challenge. While these protective Th1EFF cells may be memory

cell derived, they possess a short-lived and infection-dependent Ly6C+ effector cell phenotype prior to challenge. Surprisingly, provision of *Leishmania* antigen-specific Th1EFF cells after establishment of the pathogen niche, even when Th1 cells were provided in large quantities, abrogated protection, Th1EFF cell accumulation and IFN-gamma production, and iNOS production by inflammatory monocytes. These findings indicate that protective Th1 immunity is critically dependent on activation of permissive phagocytic host cells by pre-activated Th1 cells at the time of infection and prior to the establishment of the pathogen niche. These observations imply that CD4 T cell-based vaccines against *Leishmania* should target cell populations that can execute their effector function in the skin at the time of, or within hours, of infection.

Keywords CD4 Th1 CELLS; MONOCYTES; VACCINATION; CONCOMITANT IMMUNITY; MEMORY

Funding Canadian Institutes of Health Research

S30-03: TYPE I INTERFERONS SUPPRESS ANTI-PARASITIC IMMUNITY AND CAN BE TARGETED TO IMPROVE TREATMENT OF VISCERAL LEISHMANIASIS

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Leishmaniasis encompasses a spectrum of disease ranging from localised cutaneous lesions to visceralising, systemic forms. The role of type I IFNs in this disease is still unclear, and likely differs depending on the causative species and type of disease. Type I IFN production and their effects are highly context-specific in regards to both local tissue microenvironment and disease setting. Nearly all cells have the capacity to produce type I IFNs, including fibroblasts, endothelial cells and leukocytes. Previous work in models of viral infection reported an association between type I IFNs and the transcription factor signal transducer and activator of transcription (STAT)1. STAT1 is activated following recruitment of the Janus Activated Kinases (JAK)1 and 2 to the type I IFN receptor. STAT1 can mediate type I IFN suppressive functions in these models via induction of IL-10 production and subsequent downregulation of IFNγ receptor on NK and T cells. A similar immunosuppressive mechanism has been postulated in tuberculosis. However, the impact of type I IFNs on IL-10 production in parasitic disease is less clear. Here, I will discuss how type I IFNs contribute to *Leishmania donovani* persistence via suppressing Th1 cell development and promoting IL-10 production by these cells. I will also describe the identification of a licensed, small molecule JAK1/JAK2 inhibitor (ruxolitinib) that can overcome this immunosuppression, thus showing the therapeutic potential of targeting type I IFN signaling to improve anti-parasitic immunity in VL patients.

S30-04: PATHOGENIC CD4⁺ T CELLS ASSOCIATED WITH BONE MARROW DYSFUNCTION IN EXPERIMENTAL VISCERAL LEISHMANIASIS.

Paul M. Kaye

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Whilst T cells play a vital role in host protection against *Leishmania* infection, they are also a major mediator of the immunopathology that characterises both the local and systemic response to infection. In experimental models of visceral leishmaniasis, immunopathology has been most extensively studied in the spleen and liver and is characterised by hepato-splenomegaly with associated changes in immune and stromal tissue architecture. In contrast, though a site of infection, changes in the bone marrow microenvironment have been less well documented, despite evident anaemia, pancytopenia and thrombocytopenia associated with human disease. This presentation will review our current understanding of the changes to the bone marrow immune and stromal landscape associated with *L. donovani* infection of C57BL/6 mice and present further characterisation, based on single cell RNA-seq analysis, of CD4⁺ T cells that are associated with driving bone marrow dysfunction.

S31. RESERVOIRS OF LEISHMANIASIS

S31-01: WILD AND DOMESTIC RESERVOIRS OF *Leishmania* spp. IN BRAZIL

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Leishmania spp. are a diverse group of parasites in the Americas, where over 10 species can infect animals and humans. Brazil is the largest focus of both visceral and cutaneous leishmaniases in the American continent. These diseases are widespread within the Brazilian territory, occurring in different biomes (Amazon, Cerrado, Atlantic Forest, Caatinga, Pampa and Pantanal). A plethora of phlebotomine sand fly species are involved in the transmission of various *Leishmania* spp. and there is compelling evidence showing that multiple animal reservoirs are involved in the maintenance of these parasites in Brazil. Further studies are needed however to better quantify the actual importance of these animal reservoirs for the enzootic and zoonotic cycles of various *Leishmania* spp. in Brazil and elsewhere in the American continent.

S31-02: *Leishmania* vs *Sauroleishmania*: ROLE OF REPTILES IN THE EPIDEMIOLOGY OF LEISHMANIASES

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Leishmania parasites (subfamily Leishmaniinae, family Trypanosomatidae) include the subgenus *Leishmania* (with more than 20 species pathogenic to mammals) and *Sauroleishmania*, mainly associated to reptiles. The latter are transmitted by sand flies of the genus *Sergentomyia*, which are considered non-pathogenic and restricted to the Old World. Even before the first description of the type species of *Sauroleishmania* (i.e., *Leishmania tarentolae* Wenyon, 1921) from the Moorish gecko (*Tarentola mauritanica*) in Algeria, reptiles were thought to have a possible role as reservoirs of a zoonotic disease called Biskra boil, caused by *Leishmania* spp. Indeed, at the moment of the first isolation of *L. tarentolae*, *T. mauritanica* geckoes were suspected to be the reservoirs of cutaneous leishmaniasis caused by *Leishmania tropica* and/or *Leishmania major*. In addition, other *Sauroleishmania* species were thought to be causative agents of cutaneous leishmaniasis, known as oriental sore. For example, *Leishmnania adleri* was isolated from the blood of *Latastia longicaudata* lizards in Kenya, and was believed to be a strain of *Leishmania donovani.* Further studies did confirm the pathogenic effect of *L. adleri* as causative agent of cutaneous leishmaniasis in rodents and even in humans. It was then hypothesized that interactions between mammalian and reptilian *Leishmania* (i.e., *L. tarentolae* in mammals, *L. donovani* in reptiles), could ultimately result in partial dilution of species, immunization and eventually protection, within the two sister clades. Additional attempts were made to identify and isolate *Sauroleishmania* from endemic areas of human and canine leishmaniasis with new isolates of *L. tarentolae* from France and Italy. In particular, *L. tarentolae* is widely distributed and may infect saurian reptiles from the Gekkonidae (i.e., *Mediodactylus kotschyi*, *Tarentola annularis*, *T. mauritanica*) and the Lacertidae (i.e., *Podarcis filfolensis*, *Podarcis siculus*)

families in the Mediterranean context. Isolates of *L. tarentolae* are widely used for their potential biotechnological applications (e.g., recombinant protein production, candidate for vaccines). However, *Sauroleishmania* were largely disregarded by the scientific community for long time and the subject on the possible interaction of *Sauroleishmania* with pathogenic *Leishmania* was left behind after the 1980's. It was not until an incidental finding of *L. tarentolae* DNA in a human mummy from Brazil, that the possibility of *Sauroleishmania* infection/exposure in mammals regain attention and spurred the scientific interest. *Leishmania tarentolae* was detected through nested-PCR in humans and sand flies (i.e., *Phlebotomus* and *Sergentomyia*) in central Italy. Additionally, the likelihood of infection by *L. tarentolae* in mammals was further serologically and molecularly confirmed both in humans, and in sheltered dogs in southern Italy. Moreover, the finding of *Sergentomyia minuta* (natural vector of *L. tarentolae*) as the most abundant species in canine leishmaniasis endemic areas, further suggested the possibility of mammalian exposure to *L. tarentolae*, also considering the feeding behavior of this sand fly species on humans and dogs. On the other hand, capability of pathogenic mammalian-associated *Leishmania* to infect reptiles was studied in the late 1960s and 1970s and ultimately overlooked, mainly given the physiological differences between mammals and reptiles. Nevertheless, experimental infections of reptiles with mammalian-associated *Leishmania* species was described, and later confirmed by molecular detection of various *Leishmania* species (i.e., *L. donovani*, *L. tropica*, *Leishmania turanica*) in saurian and snakes in China. Moreover, *Leishmania infantum* was molecularly detected in lizards in areas of canine leishmaniasis in southern Italy, in sympatric occurrence with *L. tarentolae.* These molecular findings suggest the interaction between both sister-clades, yet, new isolates are needed to fully understand the natural development of *Sauroleishmania* in reptiles as well as in mammals. Similarly, beyond molecular identification, isolation of pathogenic *Leishmania* form reptiles (e.g., *L. infantum*) is necessary to unravel the role of ectothermic tetrapods as reservoirs of mammalian leishmaniases.

Keywords *Leishmania; Sauroleishmania;* REPTILES; *Leishmania tarentolae; Leishmania adleri;Leihsmania infantum*

S31-03: OTHER RESERVOIRS OF *Leishmania* (*Leishmania*) *infantum* IN EUROPE AND THEIR ROLE IN THE CONTROL OF CANINE AND HUMAN LEISHMANIASIS

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Though dogs represent the main reservoirs of *Leishmania infantum* worldwide, the parasite has been diagnosed in many other classes of mammals (e.g., rodents, marsupials, and carnivores). The role played by other animal species as reservoirs of *L. infantum* also depends on a complex chain of factors in different ecological contexts (e.g., sand fly vector density, species composition and blood feeding preferences, and length of transmission season). In addition, while cats, dogs, and humans have been demonstrated to act as a source of *L. infantum* infection for phlebotomine sand flies, studies about other animal species are scant and mainly inferred by the delineation of the blood source. For example, both the black rat (*Rattus rattus*) and the Iberian hare (*Lepus granatensis*) have been demonstrated to infect sand flies. This observation was also corroborated by the high prevalence of infected black rats found in Sicily (up to 45%) or in Montecristo, Tuscany (15.5%), being the latter a small island dog-free because of conservational reasons. Another paradigmatic example is represented by the Iberian hare, which has been implicated in one of the most important outbreaks of leishmaniasis (2009-2012) in people frequenting a suburban park near Madrid (central Spain). The prevalence of CanL did not vary in dogs from the same area, and conversely, up to 45% of hares sampled were infected by *L. infantum*. In addition, naturally *L. infantum*-infected hares were infectious to *Phlebotomus perniciosus*, showing this sand fly species a high preference for hares under natural conditions. Based on some experiments of sand fly feeding preferences and on the molecular detection of *L. infantum* in red foxes (*Vulpes vulpes*), this

animal has been suggested as a putative reservoir of *L. infantum*. Further investigations should elucidate the role of wildlife in specific epidemiological contexts also considering their impact on the control of CanL. From a public health perspective, the presence of other reservoirs could reduce the effectiveness of visceral leishmaniasis control programs focused on the application of repellents on dogs.

Keywords *Leishmania infantum;* CANINE LEISHMANIASIS, HUMAN LEISHMANIASIS, RESERVOIRS, CONTROL

S31-04: SOURCE OR SINK – NOT AN EASY DECISION

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The increasing use of molecular methods in the search for *Leishmania* in wild animals has led to records of their presence in practically all terrestrial mammals and many lizards. This has led to these animals being consider as reservoirs. According to the Oxford dictionary a reservoir is "a large amount of something that is available to be used ". CDC's definition is " The *reservoir* of an infectious agent is the habitat in which the agent normally lives, grows, and multiplies ". Together these definitions imply that a reservoir is where something increases and accumulates before moving on to somewhere else.

When a *Leishmania* infection is found the animal it is considered as a reservoir. However, reservoirs of vector born parasites may or may not be capable of maintaining the pathogen. This has lead to the use of the terms "primary reservoir", that perpetuates the enzootic cycle and "secondary reservoir" that does not but contributes to transmission potential. Chaves et al., [1] introduced the terms "Source" and "Sink" for leishmanial reservoirs. They considered that enzootics are mutli-host situations that approximate metacommunities. These terms are used extensively in ecology in defining the sustainability of habitats. Under these concepts primary and secondary reservoirs are sources irrespective of the part they play in the maintenance of the enzootic cycle as both infect vectors that lead can to people becoming infected. However, "Sinks" are dead ends that do not lead to new infections and consequently there is no feedback in the cycle. This can be defined as being non-infectious to vectors. The classical method to establish infectiousness is by xenodiagnosis. This is a difficult procedure especially with wild animals so is there a molecular method that would indicate infectiousness? Attempts to do this have do far been unsuccessful as both laboratory and wild animals with negative PCRs have had positive xenodiagnosis tests and animals with positive PCRs have had negative xenos

[2]. In infectious diseases host refers to an animal or plant that is a biological refuge in which another - often parasitic - organism may dwell. Although reservoir and host are sometimes used synonymously the more correct usage is in defining an animal as a reservoir host or an incidental host. An “incidental host" is one that becomes infected when entering the enzootic’s habitat but does not remain there. In such situations man is an incidental host when he contracts cutaneous leishmaniasis. Such hosts maybe infectious but in the absence vectorial contact are sinks. Thus the definition of a sink is not limited to being non-infectious but associated to other parameters such as exposure to vectors and an infection’s longevity. An animal’s status as “Source or Sink” may change over time according to a variety of biological and environmental conditions. For instance when the vector population is high an animal may be infectious and is therefore a source. However, if the infection is short lived and disappears when the sand fly population is low or absent it does not contribute to any feedback in the on-going cycle and is thus a sink. The absence of information on the course of a host’s infection and the dynamics of the related vector populations makes it impossible to identify an animal in which parasites have been found as being either a “Source or Sink”. This can only be done through longitudinal field studies. Unfortunately, with today’s financial restrictions it is difficult to find support for such work.

S32. GENOMICS AND EPIDEMIOLOGICAL SURVEILLANCE

S32-01: GENOME DIVERSITY IN NEOTROPICAL *Leishmania*: COLONIZATION AND HYBRIDIZATION

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Colonization and hybridization of *Leishmania* species are emerging public health concerns, because both processes have the potential to produce a whole range of adaptive phenotypes implicated in mechanisms of key pathogenic and transmission traits, including parasite-host interactions, geographic and ecological expansion, drug resistance. South America comprises an enormous fraction of global biodiversity, representing one of the most species-diverse regions in the world. Diversity of mammals and sandflies likely contribute to the evolution, speciation and diversification of *Leishmania* parasites, especially in this part of the World. The evolutionary relationships within the genus *Leishmania* and its origins are the source of ongoing debate, reflected in conflicting phylogenetic and biogeographic reconstructions. The Supercontinent hypotheses, a version of the Multiple Origins hypothesis, indicate that the genus *Leishmania* evolved at least 90–100 million years ago. According to this scenario, separate *Leishmania* clades emerged prior to, and during, the breakup of Gondwana. The level of diversity between *L*. (*Viannia*) and *L. (Leishmania*) subgenera has been considered a result of vicariance after the parting of South America and Africa. Except for importation of *L. infantum* to the American Continent during the colonization processes, only the migration of the ancestral of *L. (L.) mexicana* complex back to the New World is supported by this hypothesis. It was proposed that this migration has occurred via the Neartic and Beringia during the mid-Miocene when temperatures were warm enough for sandfly survival. This timing is roughly consistent with the Supercontinent hypotheses, showing that New World *L. (Leishmania)* diverged approximately 30 mya. Here we will focus in two important scenarios impacting human leishmaniasis in South America, emphasizing the spread of Leishmania species in Brazilian regions:

1. *Leishmania* (*Viannia*): a group of parasites arising from an evolutionary mechanism, i.e., consequence of the distribution of an ancestral species fragmented into distinct areas, due to the emergence of a natural barrier. This emerged group evolved in the new continent, a geographic area with huge diversity of mammals and sandflies, which certainly impacted in the evolution, diversification, and speciation processes of this subgenus. At least six *L*. (*Viannia*) species occur in Brazilian regions, but only *L. braziliensis*

causes human diseases all over the Country, in distinct ecotypes (although geographic distribution of such species are mainly based on human disease detection). Although not frequent, intra and inter-species hybrids are observed in this group. Various analytical approaches revealed different *L. braziliensis* population in South American Continent. The *L braziliensis* population circulating in regions of sympatry with other *L. (Viannia)* species, like in the Brazilian Amazon region, is closer related to other *L. (Viannia)* species, and significantly differs from those *L. braziliensis* from the Atlantic region, for example. Most of the species from this subgenus (if not all), adapted to the Amazon region, had strains detected as positive for an RNA virus (*Leishmania* RNA Virus 1 – LRV1). Interestingly, no LRV has been detected so far among *L. braziliensis* population from the Atlantic coast. Some studies suggest an ancient acquisition of LRV by these *Leishmania (Viannia)* species. The lack of a detectable infectious phase of LRV suggests a long-lasting relationship between the virus and the parasites, but it still unclear if LRV has a symbiotic association with *Leishmania* or if represents a virus parasitizing a parasite. Phylogenetic findings suggested that LRV acquisition by *Leishmania* parasites occurred prior to the divergence of Old and New World *Leishmania* parasites, but the finding of LRV in the monoxenous genus *Blechomonas* indicates an ancient interaction with the Trypanosomatid family. More recent studies have point out to co-evolution of *L. (Viannia)* parasites and LRV1. This process might be linked to *L. braziliensis* evolution, diversification and dispersion of LRV1-free parasites to different regions, as in the Brazilian Atlantic coast.

2*. L*. (*Leishmania*) *infantum*: this is a species recently introduced to the American continent by European settlers, probably by infected dogs as asymptomatic carriers. Biological invasions are global phenomena with widespread ecological and evolutionary implications, creating unique opportunity for extreme evolutionary transformation. The compatibility between *L. infantum* and Lutzomyia species was essential to establish long-term association and is likely the reason for the great success of this non-native species in establishing novel populations across different areas. The recent reports of genetic exchanges together with demographic history as essential mechanisms in shaping *L. infantum* diversity in the New World,

challenges the view of a scenario of homogeneous population of *L. infantum* in the Americas. These findings raise questions on the different sources and yet unknown extent of *L. infantum* diversity in the New World. There is extensive evidence of genetic exchange among *L. infantum* parasites in Brazil, pointing to the role this event has in the spread of epidemiologically relevant traits. Hybridization occurs frequently at Secondary Contact zones and possibly mating is generally common and perchance beneficial, in non-native and/or bottlenecked groups.

An important characteristic of the *L. infantum* parasite population circulating in Brazil is the presence of strains either carrying or not a >12 kb deletion at the chromosome 31. Deletion-carrying strains (Del) occur abundantly in both the country's North and South. Non-deletion genotypes (NonDel) commonly appear in the northern state of Piauí and in the southwestern state of Mato Grosso do Sul. Presence of NonDel isolates with high homozygosity and low (chromosome-specific) divergence to Del strains in other regions of Brazil, e.g., in Piauí and Maranhão might be explained by genetic exchange between closely related Del and NonDel strains, followed by further inbreeding and/or mitotic haplotype selection.

Important questions: Could distinct local selection pressures, like different invertebrate hosts, contribute to the success of a separately introduced parasite population in Mato Grosso do Sul? Might this population, and other unobserved populations, involve importations from different colonial empires, e.g., the French or Spanish, aside from the Portuguese? Does the widespread distribution of Del genotypes in the Americas reflect fitness advantages within the parasite's narrow host/vector spectrum in the New World? The above-mentioned scenarios advise a reflection on the relevance of multidisciplinary studies aiming to understand the consequences of Colonization and hybridization of *Leishmania* parasites. Such approach would likely assist in the management and prevention of disease.

S32-02: LEISHMANIASIS IN SRI LANKA: PARASITE AND HOST DETERMINANTS

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Leishmaniasis is a complex infection that impose a heavy burden on many developing countries, including Sri Lanka. The type of manifestations and the severity of the disease are dependent on many factors including the species of parasite, the host, region of endemicity, socio-economic status and the accessibility to health facilities. The predominant clinical form found in Sri Lanka is cutaneous leishmaniasis (CL) with a dermotropic variant of *L. donovani* as the causative agent. Skin lesions manifest as non-itchy, non-tender papules, nodules, ulcers or plaques. The first line of treatment is intralesional stibogluconate weekly injections. The disease often results in disfiguring scars on exposed areas of skin, such as the face with associated social stigma. The burden on the health sector in managing such cases is considerable, particularly due to the prolonged treatment. Detailed investigations looked into possible host and parasite determinants of *L. donovani*-induced CL in Sri Lanka. Demographic and clinical details of parasitologically-confirmed CL patients (n=669) were recorded from 2015 to 2019 through an interviewer-administered questionnaire. Patients' treatment response to weekly intralesional sodium stibogluconate injections were monitored through follow up until 20 weeks of starting treatment or complete healing of lesion(s). Patients who required over 12 doses of weekly SSG were categorized as poor SSG responders (CL-PR). Tissue sections were prepared from punch biopsy specimens from lesions, stained and examined for histological appearance. In situ cytokine expression of T- helper 1 (Th1) and T- helper 2 (Th2) cytokines, namely interferon (IFN)-γ, interleukin (IL)-12A, tumor necrosis factor (TNF)-α, IL-4 and IL-10 in lesion tissue were assessed by real-time RT- PCR. Parasites were isolated in culture, DNA extracted from 39 cutaneous leishmaniasis

patients (CL-SL); 19 of whom were poor responders to antimony (CL-PR), and two visceral leishmaniasis patients (VL-SL) were sequenced on an Illumina MiSeq platform. Detailed analysis and sequence comparisons were made between groups of CL and visceral disease (VL)-causing *L. donovani*. Phylogenetic analysis was done also using sequences of *L. donovani* with different origins and other *Leishmania* species. Gene expression studies were performed using RNA sequencing and transcriptomic analysis using promastigotes and amastigotes from a subset of patient isolates (n=10). Healing required 7 to 20 doses of weekly intra-lesional sodium stibogluconate (mean=12.2±0.62; treatment duration up to 4 months). The epidemiological or clinical features (age, gender, lesion type, size, location, lesion duration before starting treatment) did not significantly influence the treatment response ($p>0.05$). Patients with heavy parasite loads required more than 13 weekly doses for cure ($p=0.001$). Intensity of inflammation ($p=0.008$), number of macrophages ($p=0.001$) and epidermal atrophy ($p=0.033$) were associated with higher parasite loads. Granuloma formation and epidermal acanthosis were features of low parasite loads. Higher parasite loads together with epidermal acanthosis indicated longer healing times. Marked up regulation of the Th1 cytokine IFN-γ and down regulation of the Th2 cytokine IL-4 was seen in patients compared to healthy controls. Tissue expression of IFN-γ and TNF-α were elevated in lesions that presented later than 6 months from the time of onset, while IL-4 expression was more prominent in lesions that responded poorly to antimony therapy. Both chromosome and SNP profiles showed CL-SL and VL-SL to form two distinct groups with CL-PR also separating out from the rest. Interesting observations were also evident through phylogenetic analysis. The expected heterozygosity was much higher in VL-SL. Chromosome aneuploidy was seen in both CL and VL sequences but was more frequent in CL-SL. There were marked differences in copy numbers of many genes between the two groups. Genes of functional significance e.g. amino acid and energy metabolism, also differed between groups. Several genes associated with antimony resistance were observed in higher copy numbers in the CL-PR group. The gene expression profiles were different between CL-SL and CL-PR and also between promastigotes and amastigotes of *Leishmania*. Histology findings may be used to predict delayed responders to treatment which can thus be applied to monitor patients more closely. A prominent

Th1 response appears to support resolving of lesions, whereas a Th2 biased milieu tends to favor poor responsiveness to antimony and delayed lesion healing in *L. donovani* infections in Sri Lanka. Parasite genomic variations that occur at chromosome and gene level and possible hybridization events appear to influence differences in tropism as well as response to treatment.

S32-03: DIRECT SEQUENCING IN HOST TISSUES FOR GENOMIC SURVEILLANCE OF LEISHMANIASIS

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Major control programs targeting leishmaniasis are ongoing, and they resulted in significant reduction of disease burden in several endemic countries. However, their long-term success is jeopardized by decrease in attention due to political decisions, and change in priorities, *eg.* the recent Covid19 pandemics. Moreover, these programs are confronted with several challenges such as climatic changes, human migrations, drug resistance, the occurrence of untreated asymptomatic individuals, animals as reservoirs and super-spreaders or the natural fluctuations of the disease. As a result, new local leishmaniasis outbreaks continue being reported. Molecular surveillance by genotyping may provide information of highest relevance for control programs, such as (i) following the evolution of epidemics in time and space, (ii) characterization of new transmission cycles, (iii) outbreak studies and source identification, (iv) detection of new variants with new clinical features, (v) discovery of genetic markers of clinically and epidemiologically relevant traits. Currently, no molecular surveillance exists for leishmaniasis, despite of the existence of suitable technologies. Molecular surveillance can be carried out in a targeted way, where a defined, limited number of DNA fragments are being analyzed or in an untargeted

way, where whole genome of the parasite is looked at. In this talk, the approaches to apply whole genome sequencing (WGS) to study *Leishmania* genome directly in host or vector tissues will be discussed. Direct sequencing offers a significant added value in comparison to sequencing of isolates as -among others- it avoids genotype selection biases related to parasite isolation (bottleneck) and in vitro maintenance (fitness). However, direct sequencing is challenging because of the small proportion of *Leishmania* DNA in a massive amount of host DNA. We will review briefly the methods that can be used to enable the analysis of parasite genome in host/vector tissue (removal of methylated DNA, selective whole genome amplification (SWGA), targeted genome capture and Nanopore adaptive sequencing). Finally, we will describe progress we made to apply and optimize these methods to study genomes of various *Leishmania* species (including *L. donovani*, *L. braziliensis*, *L. tropica* and *L. aethiopica*) directly in experimental and clinical samples.

S32-04: METAGENOMICS OF *Phlebotomus papatasi* AND *Lutzomyia longipalpis*

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Sand flies serve as vectors for several established, emerging and re-emerging infectious agents. As important vectors of human disease, phlebotomine sand flies are of global significance to human health, transmitting protozoan, bacterial, and viral pathogens, the most devastating which is leishmaniasis. Here we assessed the metagenomes of two different phlebotomine sand flies, *Phlebotomus papatasi* and *Lutzomyia longipalpis*. We took three approaches to characterizing the microbiome of these two species. First, we characterized the gut microbiome of both species by Illumina sequencing of 16S RNA genes. DNA was isolated from pools of 20 females that were sugar-fed, 2 and 12 days after feeding on uninfected rat blood or *Leishmania* -infected blood. Second, shotgun microbiome Illumina and long-read PacBio sequencing was also performed on the same midgut dissections. Assemblies were performed using a combination of long-read (Falcon) and short-read (Ray Meta) and annotations were performed using the ShotMAP. Lastly, microbiome species were annotated from DNA sequences of individual *P. papatasi* females from Afghanistan, Tunisia, and Egypt and *Lu. longipalpis* males from five different populations in Brazil (Jacobina, Marajo, Sobral 1S, Sobral 2s, Laphina) and the microbiome diversity between populations was assessed.

S33. EXPERIENCE WITH mHEALTH AND LEISHMANIASIS

S33-01: mHEALTH FOLLOW-UP OF CUTANEOUS LEISHMANIASIS PATIENTS: A MIXED METHODS COMMUNITY-BASED IMPLEMENTATION STUDY.

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Cutaneous Leishmaniasis (CL) is a global health problem. Assessment of treatment outcome is a challenge because it should be done at day 90 for early response, or day 180 for final outcome. In Colombia and other countries, treatment effectiveness, as opposed to efficacy, is unknown. An effectiveness-implementation sequential explanatory hybrid design type 2 using mixed methods was performed in two rural communities of Colombia. A quasi-experimental study with historical control (standard of care) was designed to estimate the effectiveness of community-based intervention using the Guaral+ST mobile application (app). This app was used to monitor treatment adherence, adverse drug reactions, and therapeutic response. Three implementation outcomes were evaluated: acceptability and usability by qualitative methods, and fidelity by quantitative methods. One hundred-five patients were included for evaluation of effectiveness: 57 in the intervention (app) group, and 48 in the

standard of care group. Additionally, 24 community health leaders, health workers and patients participated in qualitative evaluations. The intervention significantly increased the proportion of patients having follow-up of therapeutic outcome at day 90 and 180 after initiating treatment, from 4.2% (for standard of care) to 82.5% (intervention with the app, $p<0.001$). The proportion of patients having a record of treatment adherence, adverse drug reactions and therapeutic response also increased significantly ($p<0.001$). Fidelity to the intervention strategy using the app was between 70 -100% for four components evaluated. The app was highly accepted at three levels: community health leaders, health workers and patients. These stakeholders perceived that the app improved case identification and follow-up of patients, that it met a public health need, and that it was suitable for rural areas. App usability was assessed positively, with its step by step design and illustrations facilitating its use. However, real-time transmission of data was affected by low connectivity. This community-based intervention using the Guaral+ST app for follow-up of CL patients was effective, acceptable, and easy to use, in a remote rural area. The app facilitated access to health care and may also contributed to reducing the knowledge gap regarding treatment effectiveness in the region.

S33-02: LEISHCARE®: A SOFTWARE DESIGNED FOR THE MANAGEMENT OF INDIVIDUALS WITH LEISHMANIASES*

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The aim of this speach is to describe a smartphone app aimed at healthcare professionals who work in areas endemic for visceral and tegumentary leishmaniases, and to report the user's perception of the app in these areas. The software, called LeishCare®, has the following features: data registration, image filter to record the evolution of skin lesions using photos, calculation of a score set to identify the risk of death from visceral leishmaniasis, and guides to the diseases. LeishCare® has been made available to healthcare professionals in endemic municipalities in Brazil, and the perception of potential users was evaluated at baseline and after 6 and 12 months. In the first meeting, 96 (94.1%) of the 102 professionals who knew the app reported positive expectations for its use. The installation of LeishCare® on the individual device and the evaluation of user perception were completed in 6 months with 16 users and in 12 months with 20 users. More than 90% of the professionals evaluated in both assessments found the information of the app useful. The features related to the calculation of visceral leishmaniasis severity score, and the guides to leishmaniases were the most frequently accessed. Users reported competence gain attributed to the app for all items evaluated. In conclusion, LeishCare® has been found to be a promising tool to help healthcare professionals in endemic areas with leishmaniasis management. *Published in Am J Trop Med Hyg. 2020 Aug;103(2):909-916. doi: 10.4269/ajtmh.19-0178.

S34. EMPOWERING PEOPLE WITH CUTANEOUS LEISHMANIASIS THROUGH INTERDISCIPLINARY RESEARCH AND COMMUNITY-BASED INTERVENTIONS (ECLIPSE)

S34-01: EMPOWERING PEOPLE WITH CUTANEOUS LEISHMANIASIS: AN INTRODUCTION TO THE ECLIPSE PROGRAMME

Helen Price[1], Suneth Agampodi[2], Paulo Machado[3], Leo Pedrana[3], Leny Trad[3], Kosala Weerakoon[2], Lisa Dikomitis[4]

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In this presentation I will introduce the interdisciplinary ECLIPSE programme and its aims. ECLIPSE is a four-year £4.6M healthcare programme funded by the UK's National Institute for Health Research (NIHR), which aims to improve the CL patient journey and reduce stigma in the most underserved communities in Brazil, Ethiopia and Sri Lanka. ECLIPSE brings together leishmaniasis expertise in an international, cross-cultural, multidisciplinary team of over 60 researchers, including anthropologists, parasitologists, clinicians from different medical specialties, psychologists, disease specialist and public health researchers. The ECLIPSE team are working towards a patient journey that is holistic, patient-centred and mapped on a biopsychosocial model of CL. Two interventions will be co-developed, implemented and evaluated in each ECLIPSE country, aimed at promoting early diagnosis and treatment seeking behaviour, decreasing social isolation and stigma, empowering CL-endemic communities and improving treatment pathways. The ECLIPSE team are using a range of qualitative and quantitative methods and anthropological

theories to gain in-depth understanding of people, communities and healthcare professionals experiences and views on the effects of CL on the daily lives of those affected, the barriers to seeking healthcare, obtaining accurate, early diagnosis and receiving effective treatment. The insights gained will inform the development of new interventions: community education campaigns to increase disease awareness and reduce stigma and training packages for healthcare professionals. The ECLIPSE team is strongly committed to involving community members in all the ECLIPSE activities in partner countries. This means that each stage of our applied health programme is conducted with community members, in line with our ethos: '*no research about us, without us*'. We recognise, value and wish to amplify the community knowledge and understandings of health and illness, and the facilitators and challenges to seeking treatment for CL. This recognition places community engagement and involvement at the heart of ECLIPSE. The communities' experiential knowledge, combined with other knowledge (such as biomedical and anthropological insights), will result in the co-creation of new knowledge which will underpin the ECLIPSE interventions.

More information on the ECLIPSE programme: www.eclipse-community.com

S34-02: THINKING BEYOND THE CLINICAL, PARASITOLOGICAL AND EPIDEMIOLOGICAL ASPECTS OF CUTANEOUS LEISHMANIASIS IN SRI LANKA

Suneth Agampodi

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Leishmaniasis in Sri Lanka is well documented for more than 100 years. The public health system of Ceylon (name used by the colonial rulers) had implemented an active reporting system of leishmaniasis in the early 20th century. These were limited to reporting of patient numbers and only a handful of scientific inquiries, which are not translated into journal articles. After the re-identification of cutaneous leishmaniasis as a major public health issue in the late 20th century, scientific investigations were greatly expanded. However, the research into leishmaniasis in Sri Lanka remained within the scope of the typical biomedical model. While the understanding of the parasite biology, evolution and pathogenesis is expanding, the human impact, behaviours, stigma and related issues were not taken into account as a part of the leishmaniasis research agenda. Empowering people with cutaneous leishmaniasis through interdisciplinary research and community-based interventions (ECLIPSE) project in Sri Lanka was based on the biopsychosocial model, exploring the untold stories of leishmaniasis patients, families and communities. The ethnographic and medical anthropological approach in understanding the community perceptions, behaviours and root causes of the morbidity and mortality related to cutaneous leishmaniasis in Sri Lankan rural communities is currently on the way. This includes exploring the patient journey as well as the lived experience of patients with cutaneous leishmaniasis. These approaches will help to propose a "people-centred" primary health care and public health approach in controlling this disease. Further, exploring the indigenous knowledge base on leishmaniasis as well as dealing with stigmatizing skin disorders are also being explored to be used as the foundation to develop culturally appropriate context-specific interventions in vector-borne

diseases. The present ongoing research agenda on clinical, laboratory and epidemiological work, which seems as in isolation from the application part will be supplemented by the proposed ECLIPSE work by bridging the gap between science and society.

S34-03: MEANINGS AND EXPERIENCES IN CUTANEOUS LEISHMANIASIS IN BRAZIL FROM A COMMUNITY PERSPECTIVE: LISTENING TO THE PEOPLE

Leny Trad, Clarise Mota, Leo Pedrana[1], Greice Viana, Felipe Rocha, Gisela Santos, Marciglei Morais

ECLIPSE Programme

This work presents and discuss some empirical data on meanings and experiences in Cutaneous Leishmaniasis (CL) in Brazil, from a community perspective. The data were provided by an ethnography and qualitative research which is been developed in three municipalities situated in the lower southern territory of Bahia, Brazil. Among the dimensions explored in our research, through ethnography in rural communities in the three municipalities, 72 interviews (with people who had had LC or live with this disease on a daily basis; health and education professionals; community leaders etc.) and meetings in the Community Advisory Groups (CAGa), the following topics will be highlighted in this presentation: socio-cultural characteristics of the territories identified as relevant to understand the local experience in CL, including environmental context and basic infrastructure; the assessment of the local health services focusing the treatment in CL, including the role of the Reference Centre of Cutaneous Leishmaniasis (CRLC), situated in one of the municipalities; traditional or community health practices. We are talking about a region within the Brazilian and North-eastern Atlantic forest, which is considered one of the most abundant forests in biodiversity in the planet. The socio-environmental characteristics of this region favour the persistence of endemic leishmaniasis, such as: Atlantic forest vegetation, humid climate with abundant rainfall, cocoa and banana cultivation. In addition to this context, there are the impacts of deforestation, precarious housing condition, and some habits that expose the population to the vector of the disease. In fact, according some CL specialist interviewed, who worked in rural areas of this region, there are evidences of relationship between

deforestation, family farming practises, and the multiplication of disease vectors in the surroundings of homes. The environmental conditions and habits of the rural population are also central to the understanding of the rapid spread of CL through two types of animal reservoirs: pet and wild. Some local habits, such as the raising of chickens, would explain the higher occurrence of CL observed among women and children. The region comprises an extensive and abundant area of Atlantic forest, with diverse landforms such as small hills that are cut by dirt roads with steep slopes, creating paths of difficult access. These paths are travelled by community health agents on foot, or with their own private means of transportation, such as motorcycles or family cars. There seems to be an absence of public transportation in the region, which was perceived by the fact that health unit professionals needed to anticipate the end of our meeting to take a ride in the doctor's car to avoid walking a long way back home on a “dangerous” road by themselves.

The Program of Community Health Agents (PCHAs) is the most important source of primary care in the rural areas studied. In several meetings of the Community Advisory Groups (CAGs) it was possible to realise the important role that Community Health Agents (CHAs) play in the territories. Everyone reported having extensive experience with CL (some people have contracted the disease, others have cared for at least one person with the disease), all of them are professionals with a keen perception to identify skin lesions caused by *Leishmania* and advise patients to see a doctor. However, the same people reported that they often feel delegitimised and devalued; it is possible to observe that these professionals know more than some other professionals in the unit, such as nurses and doctors, but at work they are subjected to power relations and hierarchies that prevent them from performing more specialised health care. Among the CHAs with whom it was possible to talk, most of them reported that they generally work alone: during home visits, they identify the injuries and advise people to go directly to the Reference Centre. Therefore, reports of late diagnosis resulting from the absence of trained professionals in the communities to identify injuries and direct them to the appropriate health service prevail. Among the reasons for the interruption of treatment are the lack of supplies (syringe, medicines), the insufficient number of professionals - “the injectors” in the

communities and their lack of training which interferes in the treatment continuity. The consequences of this in several cases result in cycles of up to 1 year of treatment that causes a prolonged interruption of work, a consequent reduction in family economic income and in family nutrition. Meanings and experiences in CL in Brazil from a community perspective: listening to the Through ethnography and meetings of the Community Advisory Groups that we held with the communities it was possible to understand how much the relationship with that territory touches aspects of personal and interpersonal biographies, involving economic, cultural, and spiritual dimensions. We note, among others elements, the survival of some community practises involving socialisation, self-attention to health, and the rescue or valorisation of rural life. This is the case of the use of medicinal plants, simply called "teas" that permeate the daily life of the elderly. This therapeutic use of plants is evoked by educators as a habit associated with the generation of their parents, now elderly. From the perspective of Well-Living, we learnt that it is only possible to understand the social, political, and cultural dimensions of a human problem – such as Cutaneous Leishmaniasis, for example, through a systemic and integral view, in relation to the whole. This means considering the relationships of communities with nature as a significant part in understanding the realities of the disease in this region, since it is known that the development of CL is deeply related to environmental and urbanisation contexts.

S34-04: INTERDISCIPLINARY TRAINING FOR COMMUNITY HEALTH WORKERS AND OTHER HEALTH PROFESSIONALS: INITIATIVES AND CHALLENGES

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Cutaneous leishmaniasis (CL) is known as one of the neglected tropical diseases with the highest impacts on the communities' health and the primary healthcare system in the (hyper-)endemic area of the South of the State of Bahia in Brazil. This is one of the research locus of the multisite and international ECLIPSE – Empowering people with Cutaneous Leishmaniasis - research and intervention project (also in Sri Lanka and Ethiopia) leaded by academic institutions of the United Kingdom – Kelee University and Kent and Medway Medical School - and funded by the National Institute for Health and Care Research – NIHR - https://www.eclipse-community.com/menu/about/. In the first phase of the project, the qualitative and quantitative evidences produced by our field research in the communities of the rural areas of Orobó, Corte de Pedra, São Paolino and Alto do Alegre, pointed to the generalized low level of biomedical knowledge on CL – determinations, causes, transmission, treatment, prevention – shared by different local actors and stakeholders with different experiences in CL treatment or CL disease – primary healthcare managers and professionals, education professionals of the rural school/escola do campo, patients or people with experience of CL, community members. At the same time, strongly emerged the hetorogeneity and complexity of the communities' knowledge on CL and the emic representations and perceptions based on the centenary experience of the disease. Based on these premisses, we propose to analyse two interventions of training courses on CL ecology we have build-up using participatory methodologies with two different target group qualified as multiplicators, in a sustainable perspective, between august 2021 and march 2022: the first with 20 people

of the/working in the rural communities totally on-line, and the second with 50 primary healthcare professionals and of the local municipality, mainly face-to-face. Due to the pandemics of COVID19, the first edition of the training course was realized entirely virtually (on-line meetings and off-line - remote activities), using ZoomR platform, while in the second one 2/3 of the meetings were faceto-face To develop the knowledge on CL from an intercultural and interdisciplinary perspective, we adopted the Problem-Based Learning pedagogy model. This permitted to implement moments of different knowledge sharing and group discussions on different issues of and perspectives on CL – ecology, epidemiologic data and social determinants, cultural dimensions and stigmatization processes, biomedical treatment and local public health barriers to CL healthcare. Between the challenges we highlight the following issues: the accessibility to the online modality for people from the rural area, as well as geographical and cost barriers for community health workersto participate to the face-to-face meetings in the urban area. The high satisfaction of the participants for all the dimensions of the course – level of knowledge, teaching methodologies, communication and relationship - linked with the increase of knowledge level measured with quantitative instrument are a positive result of the quality and effectiveness of the training proposal and pointed to the improvement of the awareness on CL of the health and education professionals and community members of these hyperendemic areas of CL.

S34-05: THE UNTOLD HISTORY OF LEISHMANIASIS IN SRI LANKA

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Leishmaniasis is a growing health issue in Sri Lanka, and a substantial number of new cases are reported annually from different regions of the island. Cutaneous leishmaniasis (CL) is the predominant clinical form found in the country and is caused by *Leishmania donovani* zymodeme MON-37. Only a few cases of confirmed visceral leishmaniasis (VL) and mucocutaneous leishmaniasis (MCL) have been reported from across the country. But the recent reports denote the possibilities of higher threat of VL and MCL in future. Though it is widely believed that the first case of leishmaniasis in Sri Lanka was reported in 1992 as in many modern-day reports, there are recently captured historical evidence to prove that the documented history of leishmaniasis, both CL and VL in particular, in Sri Lanka may extend well beyond centuries from now. Leishmaniasis is a notifiable disease in Sri Lanka since 2008, and it is now mandatory for any medical practitioner treating a patient with leishmaniasis to initiate the reporting process. This well-established surveillance system facilitates the analysis and understanding of the disease dynamics across the island, and it is pivotal to reassess the process in place to understand the areas to improve. Moreover, in 2019 the government issued guidelines on prevention and control of leishmaniasis detailing specific procedures to be followed at different levels and circumstances to alleviate the problem of CL

within the country. The epidemiology unit of Sri Lanka is identified as the main focal point for prevention and control of leishmaniasis in SL. Intricate genetic and morphometric diversity of *Leishmania* parasite as well as the sandfly vectors, and potential existence of diverse reservoir animals make the control of this disease within the local context is extremely difficult. It is imperative to explore further into these avenues for a better understanding and to implement successful control and preventive measures. Apart from biological and environmental factors, human behaviors and population dynamics play a crucial role in disease transmission and the amount of socio-economic impact of the infection. Achieving a significant disease control and subsequent elimination requires extensive analysis and exploration of these entities. Improving community awareness, vector and reservoir management, and development of effective diagnostic tools and treatment methods would be the key pillars of success.

S35. DATA FOR DECISION MAKING FOR VL ELIMINATION

S35-01: KA-MIS DEVELOPMENT AND APPLICATIONS IN THE POST-ELIMINATION PHASE

Joy Bindroo

CARE-India

Individual case data for VL and PKDL is recorded near real-time into an online application (Kala Azar MIS, or KAMIS), established for the VL elimination program by the government in India that has case lists going back to 2013 for the two highly affected states, Bihar and Jharkhand. The data is organized by village and block (sub-district) of residence and the date of diagnosis and includes basic details pertaining to modes of diagnosis and treatment as well as follow-up for at least three years. Analyses of the KAMIS data helped establish distinct patterns of incidence that showed, for instance, that around 70% of cases in any given year are detected from villages having any known case in the previous three years and that less than 25% of all villages in the affected districts have been affected since 2013. It was also shown that the risk of additional cases occurring in a village increase with the number of cumulative cases reported, reaching over 90% for villages with a cumulative case load of 15 or more cases; and that it is possible to predict the proportion of cases over any subsequent year that will arise in a previously affected set of villages. These analyses pointed to the possibility of empirically defining an outbreak in villages in these endemic regions, and also to predicting them before they became full-blown. We have attempted to evolve predictive criteria for outbreaks using accumulated KAMIS data. We hypothesised that the acceleration in incidence at the beginning of an outbreak could be used to predict the

occurrence of an outbreak. To our knowledge, there is no empirically established definition of a VL outbreak, although outbreaks get reported regularly, based on evidently unusual high incidence over a defined duration. To define outbreaks for the purpose of the analyses, we derived peak incidence velocities over any 6-month period from outbreaks identified by VL programme field staff and applied these to the cases recorded since 2017 to identify additional villages that showed a similar velocity at any time since. Further iterations using wider window periods identified more villages with periods of high incidence. We examined the cumulative incidence curves of identified, putative 'outbreaks' to identify inflexion points that indicated the beginning of the incidence acceleration and calculated the incidence velocity for the first few cases after the inflexion point for all such instances. This value was applied to the entire dataset after further adjustments, to arrive at a threshold that had a reasonable trade-off between sensitivity and specificity for identification of instances in villages that could be construed as outbreaks. Further refinements were attempted using geospatial coordinates and known village populations to improve salience. We present the methods and results of these analyses and how well the criteria predicted recent outbreaks.

S35-02: TRANSMISSION DYNAMICS: FROM EPIDEMIC CYCLES TO ELIMINATION AS A PUBLIC HEALTH PROBLEM, AND HOW TO SUSTAIN THE SUCCESS IN THE FUTURE

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Over the hundred years since *Leishmania donovani* was confirmed as the etiological agent, *Phlebotomus argentipes* as the vector and humans with kala-azar or post-kala-azar-dermal leishmaniasis (PKDL) as the primary infection reservoir, the Indian subcontinent has seen at least five major epidemic cycles of VL, peaking in the 1920s, the 1940s, 1977, 1992 and most recently in 2007. The cycles tend to last around 15 years from initial rise to eventual decline; for example, the most recent cycle started in the late 1990s and incidence had already declined substantially by 2013. The time between peaks is not fixed, ranging from 15 to more than 30 years. Hypothesized determinants of the variability include climatic conditions, outbreaks of other infectious diseases such as influenza, residual reservoir host density in the form of PKDL or undetected VL patients, and level of population immunity. In the 1950s and 1960s, reported VL incidence is anecdotally reported to have fallen to extremely low levels, although sporadic cases were documented in hospital records throughout the period. The apparent prolonged interepidemic period until the mid-1970s is hypothesized to have resulted from the impact of the blanket DDT spraying by the malaria eradication program, which was abandoned in the 1960s. We now have the most effective surveillance in the history of this disease in India, in the form of the Kala-Azar Management Information System (KAMIS). Based on these data, we can say with confidence that VL incidence is the lowest ever documented. Although IRS with an effective synthetic pyrethroid insecticide was implemented in 2015, the great majority of the decline in VL incidence antedates this intervention, and no sustained impact of IRS on sand fly density or VL incidence has been convincingly demonstrated in recent years. The decline in VL incidence had

already begun before active case detection, rapid VL diagnostics and shorter, more effective treatment regimens were introduced, but mathematical modeling suggests that the consequent shortening of symptomatic VL and PKDL duration, representing reservoir reduction, has played a major role in driving incidence to the current low levels. Nevertheless, sporadic cases and small outbreaks continue to occur, making it clear that transmission has not been interrupted. Data will be presented from an ongoing research study that focuses on patterns of and risk factors for sand fly and parasite exposure through longitudinal data and serum collections in villages with varying levels of VL case incidence. Our analyses will help to elucidate where and when exposure is occurring, and provide evidence to guide surveillance to detect case clusters as early as possible. Research is needed to better understand which preventive interventions are effective and which are not, whether we have missed potential reservoirs, and to maintain the knowledge base among providers who may see VL rarely if at all.

S35-03: PKDL AS A CHALLENGE TO VL ELIMINATION

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Post-kala-azar dermal leishmaniasis (PKDL) is a pleiomorphic dermatosis that occurs months to years after clinical cure of kala-azar in 5-15% of patients in the Indian subcontinent. Patients are not systemically ill and have been shown to be infectious to sand flies in human xenodiagnosis studies, with higher levels of infectiousness for nodular PKDL than for the macular form. PKDL is hypothesized to constitute the major reservoir of continued transmission when KA incidence is low, and to provide the means for the parasite to survive over interepidemic nadir periods. PKDL patients often have lesions for many months or even years before diagnosis and treatment. Definitive diagnosis requires demonstration of the parasite in slit skin swabs or skin snips by smear or PCR. In the absence of skin sampling, diagnoses are presumptive, based on the history of antecedent kala-azar, the presence of persistent lesions, and positive results by rK39 rapid test. Current treatment consists of 12 weeks of miltefosine. Recent reports of serious ocular complications in some patients during miltefosine treatment have called into question the safety of the prolonged drug course. Decreasing the infection reservoir due to PKDL patients requires shortening the time from lesion onset to effective treatment. PKDL cases are reported in the Kala-Azar Management Information System (KAMIS). Since 2017, active case detection (ACD) has been implemented in endemic districts of Bihar and Jharkhand; reporting of the first confirmed kala-azar/PKDL case sparks the ACD activities in the village and consists of the following activities: 1) Case searches in the household of the index case, based on the information provided by the case, followed by snowballing in the vicinity of the case's household; and 2) Fortnightly follow-up with key-informants such as rural health practitioners, ASHA, community member, etc. over a period of at least a year. In a previous analysis, we showed that ACD significantly decreased the time to diagnosis for kala-azar patients

compared to passive case detection (PCD). ACD was associated with a decrease in the proportion of patients with very long delays, defined as a period of longer than 90 days from onset of symptoms to diagnosis. Other factors associated with diagnostic delay included male sex, older age and HIV infection. We propose to apply similar methods to evaluate diagnostic delays among PKDL patients. We analyzed data from 3222 PKDL patients reported in KAMIS from 2017 to 2022; 1823 (57%) were male and the mean age was 28 years (range 2-93 years). Information on lesion types and affected body parts was available for 2535 and 2525, respectively. Lesions were reported as consisting solely of hypopigmented macules in 1842 (73%); while 202 (8%) had nodules with or without other types of lesions, and 491 (19%) had papules with or without macules. In 316 (13%) of the cases, most anatomical areas, including the face, torso, limbs, ears, neck, etc. were affected. The face was affected in 2287 (91%) cases and was the most commonly affected body part among cases with macular lesions (88%). 829 (26%) of PKDL patients were reported through ACD and 2393 (74%) through PCD. Approximately, 2/3rd of the cases were diagnosed through rK39; skin smear was used for diagnosis in 658 (20%) cases. Miltefosine, the first line of treatment for PKDL in national guidelines, was used to treat 2857 (89%) cases followed by Liposomal Amphotericin B in 246 (8%) cases. Further analysis is underway to describe the distribution of lesion duration prior to treatment start and to explore factors associated with prolonged duration. Without timely diagnosis and treatment for PKDL, the elimination program risks continued transmission and potential outbreaks. Addressing PKDL as a future reservoir will require the development of sustainable methods of detection, more specific and sensitive diagnostic testing, and a safe, shorter treatment regimen with high efficacy.

035-04: MODELLING SPATIOTEMPORAL PATTERNS OF VISCERAL LEISHMANIASIS INCIDENCE IN INDIA USING ENVIRONMENT, BIOCLIMATIC AND DEMOGRAPHIC DATA, 2013-2021

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Visceral leishmaniasis (VL) is a vector-borne disease caused by *Leishmania donovani* and transmitted through infected female *Phlebotomus argentipes* sandflies. In India, VL has been endemic in 633 subdistricts (blocks) spread over 54 districts in four states (Bihar, Jharkhand, Uttar Pradesh and West Bengal) affecting nearly 150 million people. As of 2020, the National Kala-azar Elimination Programme has achieved VL elimination (<1 case / 10,000 population/year in each 'block') in 596 blocks. There is a need for sustaining the elimination level in these blocks, while a more targeted approach is necessary to achieve the WHO 2030 target in those blocks where elimination has not been reached. In an earlier publication, applying a statistical model on surveillance data collected from the states of Bihar and Jharkhand in India, we forecasted monthly VL incidence at the block level. The model predictions may be used to help the programme for logistics management in advance. In this study, we have improved the predictive power of the model incorporating (i) environmental, bioclimatic and

demographic factors that influence VL transmission dynamics, and (ii) spatial, temporal and spatiotemporal random effects to minimize the variability unexplained by the above factors. We modelled the spatiotemporal distribution of reported VL cases for a 9-year period (2013-2021) in the states of Bihar and Jharkhand and its association with environmental, bioclimatic and demographic factors using non-parametric models with space-time interactions. A negative binomial distribution was assumed to describe the block level monthly VL cases. Initially, we fitted 46 models to a training data set (2013-2018) using the Bayesian inference via Integrated Nested Laplace Approximation (INLA) approach. The best fitting model was selected based on deviance information criterion (DIC) and was validated with a test data set (2019-2020). The model was further used to forecast VL incidence beyond the period of observations (2021-2022). We found that minimum temperature, enhanced vegetation index, population density and, isothermality played a positive role in VL occurrence. Conversely, precipitation, maximum temperature and soil moisture were negatively associated. During both training and testing periods, model predictions agree with the observed declining trends in many blocks both above and below the elimination threshold. Predictions beyond the period of observations (2021-2022) showed that the annual incidence is more likely to exceed the elimination threshold in the blocks where the reported VL incidence was > 6 per 10,000 population in 2013. Our spatiotemporal modelling framework with environmental, bioclimatic and demographic factors could better explain spatiotemporal patterns in VL incidence at block level and therefore may be used to forecast trends in incidence during post-elimination. Model predictions for 2022 highlighted the need for targeted control measures in blocks where the annual incidence was > 6 per 10,000 population in 2013 to achieve elimination.

Keywords KALA-AZAR; LEISHMANIASIS; SPATIOTEMPORAL TRANSMISSION; FORECASTING; INDIA

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S36. LEISHMANIASIS AND IMMUNOSUPPRESSION

S36-01: CLINICAL MANAGEMENT OF VL IMMUNODEPPRESSED PATIENTS

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Immunosuppression is the main individual risk factors for overt clinical visceral leishmaniasis (VL), and can also alter disease presentation and treatment response. Immunosuppression can be caused by coinfection with pathogens like human immunodeficiency virus (HIV) or by the therapeutic use of immunosuppressive drugs like tumor necrosis factor-a (TNF-a) antagonist or steroids in patients with chronic disease. Immunosuppressive conditions constitute an important challenge in *Leishmania*-endemic areas as illustrated by the *L. infantum* community outbreak occurred in Fuenlabrada (Madrid), where among the 446 cases detected between July 2009 and December 2012, 31.3% of VL cases had immunosuppressive conditions, mostly non-HIV-related (Arce A, Estirado A, Ordobas M et al. Re-emergence of leishmaniasis in Spain: community outbreak in Madrid, Spain, 2009 to 2012. Euro Surveill 2013; 18: 20546. The current management guideline for VL in immunocompromised patients is not well established. For HIV patients, the usual treatment regimen is Liposomal Amphotericin B (LAB) with a wide dosage range (20-60mg/kg total dose, usually 30-40: 3-4mg/kg per day for 10 days, the first 5 daily doses and the other 5 weekly). In this group of patients there is evidence of the benefit of continuing after treatment with secondary prophylaxis to prevent relapses, usually monthly with LAB. There is not a conclusive recommendation on when to stop secondary prophylaxis, it has been suggested that it could be suspended

with total CD4 above 200-350 in 2 consecutive determinations separated by at least 3 months, although some authors recommend maintaining it indefinitely. Regarding other types of immunosuppression, it is common practice to advise the withdrawal of immunosuppressants, although there is no recommendation on when to reintroduce them. It is recommended to treat patients with 20-40mg/kg/total dose, although there is no evidence to recommend one or the other dose. Finally, despite a higher risk of relapse being demonstrated, it is not recommended to start prophylaxis universally, as there is no evidence in this regard. Based on the experience gained in the diagnosis, treatment and follow-up of immunosuppressed VL patients that we have managed at the University Hospital of Fuenlabrada, we have designed a management protocol for the effective control of the disease, which achieves clinical cure of the patients and prevent relapse without the need to establish a universal secondary prophylaxis regimen. In addition to clinical follow-up, this protocol includes molecular diagnostic tests for leishmaniasis (PCR) and cell tests to determine the patient's specific immunity against *Leishmania*: in vitro cell proliferation assay in PBMCs stimulated with leishmanial antigens (CPA) and Interferon-gamma production in stimulated cells (IFNγ). Regarding patients with HIV-*Leishmania* coinfection, the protocol consists of treating HIV+ patients with 30-40mg/kg/total dose of Liposomal amphotericin B (LAB) following current standard recommendations. Patients should start HAART (Highly Active Anti-Retroviral Therapy) for HIV treatment as soon as possible. 2-4 weeks after the last dose of LAB, PCR, CPA and IFNγ are performed. If the patient presents a PCR-/CPA+/IFNg+ profile, secondary prophylaxis is not started, and the patient is monitored every two to three months during the first year (every 2 months in the first semester and every 3 months in the second) to confirm that this profile is maintained. If after treatment the pattern is PCR+/CPA-/IFNγ-, monthly secondary prophylaxis with LAB is started, according to standard recommendations, and it is analyzed every three months until the pattern changes to CRP-/CPA+/IFNγ+ and the prophylaxis can be withdrawn. No relapse was observed in any patient without prophylaxis who achieved the CRP-/CPA+/IFNγ+ profile and maintained HAART. In patients with VL under immunosuppression with drugs, it is advisable to withdraw immunosuppression if possible, and

standard treatment with LAB is performed at a dose of 40mg/kg total dose. 2-4 weeks after the last dose of LAB, PCR, CPA and IFNg are performed. If a PCR+/CPA-/IFNγ- profile is obtained, monthly prophylaxis is started and immunosuppression is not restarted, or retreatment is considered. If the pattern is CRP-/CPA+/IFNγ+, immunosuppression can be restarted without starting prophylaxis. It is essential to carry out follow-up every two-three months (2 months the first semester, 3 months the rest) to confirm that this pattern is maintained, since patients who remain under immunosuppressive treatment, unlike HIV patients, they can lose the profile PCR-/CPA+/IFNγ+, with a high risk of clinical relapse. In conclusion, in immunosuppressed patients treated for VL, PCR-/CPA+/IFNγ+ indicates a LOW risk of relapse, and CRP+/CPA-/IFNγ- indicates a HIGH risk of relapse after treatment, which is very useful for clinical decisions regarding treatment and prophylaxis in this group of patients.

S36-02: STUDY OF EXPERIMENTAL VISCERAL LEISHMANIASIS IN PHARMACOLOGICALLY IMMUNOSUPPRESSED MICE

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Control of *Leishmania* infection and the achievement of clinical cure depends on the development of an appropriate immune response against the parasite. The antiparasitic agents reduce parasite loads to a number that the immune system is able to control and the patient finally gets cured and becomes resistant to reinfection. Thus, being in an immunosuppressed state is a major risk factor for developing severe visceral leishmaniasis (VL). For example, co-infection with HIV increases the chances of developing VL and the treatment failure rate. The use of immunosuppressive drugs in patients with autoimmune diseases or those undergoing organ transplants is one of the most common causes of immunosuppression. In fact, during the leishmaniasis outbreak in Madrid (Spain), one of the most important *Leishmania* outbreaks in Europe, patients with this condition living there were at greater risk of infection by this parasite. Therefore, the increasing use of these therapies may have an impact on the number of cases in *Leishmania*-endemic areas. However, it is not clear how immunosuppressive drugs affect to leishmaniasis control. The range of these drugs is wide and they have multiple effects not fully understood. Consequently, clinicians face additional challenges when attempting to treat patients with leishmaniasis that are also receiving immunosuppressive treatment. First, it is necessary to decide whether the immunosuppressive treatment can be withdrawn to favor the immunological cure or not, depending on the risk of the previous pathology. Second, if the treatment

has been suspended, it should be reintroduced as soon as possible while considering the risks of therapeutic failure or clinical relapse. In order to understand the effect of the immunosuppressive drugs in patients with VL the first question to answer is whether the treatment influences the development of leishmaniasis after infection or not. The mouse model is appropriate to address this issue because it allows to study immunological features related with the mechanisms of protection and susceptibility. We evaluated the use of anti-TNF therapy, methylprednisolone (MPDN), and methotrexate (MTX) because they are among the immunosuppressants most often used to treat rheumatoid arthritis, lupus erythematous and inflammatory bowel disease. Thus, we compared the parasite load in, and immune response of, mice treated with these agents that were infected with *L. infantum*. The results showed that these treatments influence the course of infection at the first four weeks after infection but in a different manner. For example, the continued administration of anti-TNF antibodies leads to an increased liver parasite load compared to control animals and also to mice treated with the other immunosuppressants. In the mouse model, the liver experiences the highest parasite loads of any organ in the first weeks of infection (acute phase), but later these fall spontaneously. However, the presence of anti-TNF antibodies impedes natural resolution of the infection in this organ. TNF, together with IFN-γ, is a key cytokine in the activation of the infected macrophages by *Leishmania*, and it plays a central role in in the resolution of granulomas. While granuloma formation and self-cure occurs in liver, the immune response against *Leishmania* begins to fail in the spleen and leads to uncontrolled parasite multiplication in this organ and in the bone marrow during the chronic phase of the infection. Although our analysis was performed before the chronic stage, we found in the spleen of anti-TNF- and MTX- treated animals lower parasite loads that the MPDN and the control groups. T cell exhaustion and tissue damage mediated by an excessive inflammatory response lead to the remodeling of the splenic architecture, an ineffective cellular response and compensatory mechanisms, such as IL-10 production, that promote parasite persistence. Thus, some immunosuppressants could reduce this host detrimental effect during the acute phase and delay the dysfunction of the spleen. However, their effects during the chronic phase should be further analyzed. In this sense, parasite loads increased in the bone marrow of anti-TNF- and MTX -

treated animals four weeks after infection. The resolution of *Leishmania* infections depends on a good T $CD4^+$ response. Following infection, anti-TNF- and MTX- treated animals showed a remarkable reduction in the number of peripheral $CD4^+$ T cells to below normal limits, a much greater reduction than seen in control and MPDN groups. Leucopoenia regularly occurs with anti-TNF treatment, and MTX, which is an antagonist of folic acid that is used for leukemias, autoimmune and inflammatory conditions, reducing the multiplication of lymphocytes. Immunosuppressant treatments also influence in a different manner the quality of T cell-mediated immune response to *L. infantum.* A specific Th1 response is necessary for resolving *Leishmania* infections; the cytokines produced, especially IFN-γ, mediate this protection. While the Th1 response in stimulated splenocytes from control animals is elicited exclusively by IFN-γ-producing $CD4^+$T cells, the MPDN treatment induced a response mediated by $CD4^+$ and $CD8^+$ T cells that produced different types of cytokine (IFN-γ, IL-2, TNF). However, MTX-treated animals showed an increase in IFN-γ^+-$CD4^+$ and TNF^+-$CD4^+$ T cells, and IL-2^+-$CD8^+$ T cells in response to *Leishmania* antigen. Interestingly, anti-TNF treatment is associated with cellular responses exclusively mediated by TNF-producing-$CD8^+$ T cells. The *Leishmania*-specific total IgG titer increased from the beginning of the infection until the end of Week 4, but only significantly so in the anti-TNF-treated animals. The humoral immune response to *Leishmania* is not protective; rather, it is a sign of active VL in both patients and experimental animals. Of note, MTX seems to inhibit IgG responses because treated animals returned low IgG titer and it did not increase during the infection. The analysis of the subtypes of IgG in experimental models is useful for determining the kind of response underway. MPDN-treated mice produced IgG1 antibodies, which are associated with Th2 responses; this was not seen in any other group. Nevertheless, despite a marked IgG1 antibody production, MPDN did not affect severely the anti-*Leishmania* response in the liver, spleen or bone marrow in a short-term infection. Once confirmed that treatment with some immunosuppressive drugs influence the parasitological and immunological evolution of the *Leishmania infantum* infection in different manner, the next important question to address is whether these treatments can affect the effectiveness of antiparasitic drugs. Importantly, parasite loads in immunosuppressed mice receiving anti-TNF

antibodies or MTX and further infected with *L. infantum* were low after administration of a 21-day treatment with the antileishmanial drug Glucantime® at week 6 post-infection. No differences in parasite burden were found between control and immunosuppressed animals, and parasitological cure was achieved in all groups. Thus, no sign of treatment failure was concomitant to immunosuppressive treatment with anti-TNF antibodies or MTX. After cure, infected animals and patients usually acquire strong and long-term protection against reinfection. However, immunological data indicate that this may not be the case in presence of immunosuppressants. An increased splenomegaly and cellularity were observed in MTX-treated animals that were related to higher frequencies of effector $CD8^+$ T cells, along with higher frequencies of IFN-γ^+-$CD4^+$ T cells. Notably, higher production of IL-4 and IL-6 was observed in MTX-treated animals, which may impede the establishment of an appropriate anti-*Leishmania* Th1 response in case of relapse or reinfection. Nevertheless, the highest iNOS expression (thus, production of anti-*Leishmania* agent NO) was found in MTX group. Animals treated with anti-TNF antibodies showed higher IgG titer, although it decreased after the treatment with Glucantime® and, interestingly, the IgG profile changed from IgG1 to Th1-related IgG2a antibodies. However, anti-TNF therapy abolish TNF-related Th1 responses and, despite parasitological cure and an IgG2a polarization after treatment, the cellular response against *Leishmania* antigens produced higher levels of anti-inflammatory IL-6 and IL-10 cytokines that are not be protective against *L. infantum,* together with lower IFN-γ/IL-10 ratio. Our investigation clearly suggest that the immunosuppressant agents can influence the immune response to *Leishmania* infection and the course of disease, even after a short immunosuppression period. Remarkably, treatment with anti-TNF antibodies at their clinical dose impedes natural resolution of the infection in the liver of mice infected with viscerotropic species, which can be considered a site of acute self-resolving infection having similar features than asymptomatic individuals that may have higher risk of progressive visceral disease in otherwise asymptomatic infections. Interestingly, it has been reported that MTX has an antiparasitic effect against *Plasmodium vivax* and *Leishmania tropica* via its reduction of folate availability. However, it is important to note that *Leishmania* promastigotes has been described as having the potential to generate rapid resistance to

methotrexate, which may trigger a worsening of the disease or cross-resistance with anti-*Leishmania* drugs. It is therefore necessary to monitor both aspects over time in order to better establish the relationship between *Leishmania* and the immunosuppressant MTX. Because immunosuppressive therapy is commonly prescribed as a combination of two or more drugs, the effect of different combinations of immunosuppressants should be also investigated. Importantly, anti-TNF antibodies and MTX treatment may not interfere directly with the efficacy of Glucantime®, however, a suboptimal immune response associated with these treatments may increase the risk of relapse or reinfection. In addition, other, anti-*Leishmania* drugs different from antimonials should be evaluated. The immunobiology of leishmaniasis is complex and resolution of the infection depends on an equilibrium between inflammatory and regulatory responses. For that reason, the effect of immunosuppressive drugs in disease progression may be different in other forms of leishmaniasis in which the role of the immune response in the immunopathology varies, as occur, for example, in different forms of cutaneous leishmaniasis. Although many questions still remain regarding pharmacological immunosuppression and leishmaniasis, the results so far indicate that clinicians need to bear in mind the risk of leishmaniasis to patients thus treated, and choose the best immunosuppressive treatment in each case. Likewise, the use immunological and molecular diagnostic tools to detect asymptomatic individuals and opportunistic infections in the follow up of immunosuppressed patients should be considered in endemic areas.

S36-03: DEPLOYMENT-ACQUIRED ASYMPTOMATIC *Leishmania infantum* INFECTION FOLLOWED BY IMMUNOSUPPRESSION: HOW DO PATIENTS FARE?

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An interesting experiment was set up when more than a million healthy persons were traveled from non-endemic United States to visceral leishmaniasis-endemic Iraq (and Afghanistan) and then returned to the US. Subsequent surveillance allowed us to identify that there was a rate of 19.5% asymptomatic visceral leishmaniasis related to Iraq travel. Individuals with three types of post-deployment immunosuppression, specifically use of tumor-necrosis factor inhibitors, those suppressed post solid organ transplant, and HIV infection were enrolled and tested for asymptomatic visceral leishmaniasis and had a detailed medical record review including pertinent laboratory, radiology and clinical signs/symptoms that might represent the spectrum of illness of visceral leishmaniasis spanning since initiation of immunosuppression. Our results will be compared to results of published studies among individuals in endemic regions where potential re-exposure, exposure once immunosuppressed, and less defined onset of *Leishmania* infection may confound determination of re-activation versus new infection.

S37. LEISHVET: ANIMAL LEISHMANIOSIS: IS A CHANGE OF MIND NEEDED?

S37-01: CANINE LEISHMANIOSIS CAUSED BY *Leishmania tropica* AND *L. major* – DIFFERENCES FROM *L. infantum* INFECTION

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Leishmania major and *Leishmania tropica* cause human cutaneous leishmaniosis in Asia and Africa with intrusion of *L. tropica* also into Greece in southern Europe. In the Middle East and Israel, these two *Leishmania* spp. are common causes of human infection with wildlife mammal reservoirs. *Phlebotomus papatasi* is the sand fly vector for *L. major*, and *Ph. sergenti*, *Ph. arabicus* and other sand fly spp. serve as vectors of *L. tropica.* Although *L. major* and *L. tropica* are considered rare causes of canine leishmaniosis, dogs have been shown to suffer from clinical disease associated with infection of these *Leishmania* spp., in Iran, Saudi Arabia, Egypt, Israel and North Africa. The most common cause of canine leishmaniosis is *L. infantum* which causes a systemic and potentially fatal disease that involves both the visceral organs and the skin. In contrast, *L. major* infection of dogs is usually manifested only as a skin disease, while *L. tropica* infection may involve the skin and also visceralize and cause a systemic disease with similar clinical manifestations to *L. infantum*. The diagnosis of canine leishmaniosis caused by *L. tropica* and *L. major* can be done by cytology or histopathology demonstrating the parasite in tissues, followed by PCR to verify the infecting species identity. ELISA serology with whole promastigote antigen is not distinctive between *L. infantum*, *L. major* and *L. tropica* and some *L. major* infections are not seropositive. Treatment of canine *L. major* and *L. tropica*

infections is carried out using the same drugs which are indicated for treatment of *L. infantum* infection including allopurinol, meglumine antimoniate and miltefosine.

S37-02: EQUINE LEISHMANIOSIS CAUSED BY *Leishmania infantum* – CLINICAL FEATURES, SUB-CLINICAL INFECTION AND DIAGNOSIS

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Leishmaniosis caused by the protozoan *Leishmania infantum* is a zoonotic infection with a very wide prevalence covering both the northern and southern hemispheres, and the Old and New Worlds, ranging from China in the east to Brazil and Central America in the west. The dog is considered the main reservoir host for human visceral leishmaniasis caused by *L. infantum,* however other domestic and wildlife animals are also infected with this species. Equine leishmaniosis caused by *L. infantum* with clinical manifestations has been infrequently described. Only a small number of clinical cases of equine leishmaniosis have been reported so far from areas where the disease is endemic in dogs and humans, and it has usually been described as self-limiting cutaneous disease in which lesions healed spontaneously within 2-6 months. The diagnosis is mainly made by cytology, histology with *Leishmania* immunohistochemistry or by PCR, since serology for *L. infantum* specific antibodies is frequently negative. *Leishmania infantum* infection in horses is possibly underdiagnosed due to the mild disease observed in the majority of clinically affected horses and its spontaneous regression. Published data from the Mediterranean area and other parts of the world indicates that horses may be infected with *L. infantum* more frequently than previously thought and may potentially also serve as reservoirs for the disease. The aim of this talk is to provide an updated overview of equine leishmaniosis due to *L. infantum* focusing on clinical and epidemiological aspects as well as diagnosis.

S37-03: EPIDEMIOLOGY OF ANIMAL LEISHMANIOSIS IN MADRID AREA AFTER THE HUMAN LEISHMANIOSIS OUTBREAK

Guadalupe Miró

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Leishmaniosis, due to *Leishmania infantum*, is a zoonotic disease which is endemic in Spain and has been notifiable when found in humans in the Community of Madrid since 1997. In dogs, notification is mandatory in some other regions. From July 2009 to March 2022, 782 human leishmaniosis cases were reported in the southwest area of Madrid, with a corresponding incidence rate of 11,6 per 100,000 people, which is considered the largest outbreak of human leishmaniosis notified in Europe. When the outbreak was detected, multiple measures were implemented focusing on domestic and stray dogs, considered the "main" reservoir, until a newly reservoir (hares and rabbits) was incriminated. Moreover, in the last decade, *L. infantum* infection has been demonstrated in other domestic and wild species of mammals: cat, domesticated ferret, wild carnivores (fox, grey wolf, Bengal tiger, genet, mongoose, Iberian lynx, European mink), lagomorphs (hares, rabbits), rodents, Bennet's wallaby, rock wallaby, orangutan chimpanzee, meerkat, South American sea lion and bats. Surveillance activities, research, and environmental and epidemiological monitoring coordinated by the Ministries of Health and Environment have been performed in the Madrid area focused on the reservoirs, the vector, and the environment, implementing different Canine Leishmaniosis Surveillance System, Lagomorphs and Vectors control plans. It is essential to highlight that an early warning strategy for human and animal leishmaniosis implies a One Health approach. Collaboration between health authorities, veterinarians, physicians, researchers, ecologists, etc. is imperative for managing this human leishmaniosis outbreak under control.

S37-04: THE SAND FLY SKIN PARASITE INTERFACE.

Patrick Bourdeau, Christine Petersen

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Leishmanioses are parasitic diseases with significant dermal trophism. The skin is an important site of infection contributing to parasite transmission to naïve sand flies. Understanding how parasitism of host skin and the related immune microenvironment supports or prevents skin parasite replication is now the focus of major investigation in the field of Leishmaniosis research. We will discuss dermal *Leishmania* parasite burden during different stages of disease, the role of skin parasitism in transmissibility to sand flies and the growing understanding of dermatoimmunology from canine leishmaniosis and other relevant systems. We will discuss epidemiology of canine Leishmaniosis, a key sentinel for human infection. We explore the association between spatial distribution and burden of parasites in the skin in driving outward transmission. Factors associated with parasite persistence in the skin are examined. We discuss systemic immunity during canine leishmaniosis and what is known about immunological correlates in the skin microenvironment. Finally, we touch on factors egested into the skin during *Leishmania* inoculation by sand flies. Throughout, we discuss factors associated with early and chronic establishment of *Leishmania* parasites in the skin and the role of the dermal immune response.

S37-05: CONCOMITANT INFECTIONS IN LEISHMANIOSIS: A FORGOTTEN PROBLEM

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Vector borne zoonotic diseases remain important public health concerns across the globe. Canine vector-borne diseases are distributed worldwide and are caused by several bacteria (e.g. *Anaplasma, Bartonella, Borrelia, Ehrlichia* and *Rickettsia*), nematodes (e.g. *Dirofilaria immitis* and *Dirofilaria repens*), protozoan (e.g. *Babesia*, *Hepatozoon* and *Trypanosoma*) and viruses (e.g. Tick-borne encephalitis virus). Furthermore, these pathogens are transmitted to dogs by different vectors, such as *Rhipicephalus sanguineus* ticks (e.g. *Anaplasma platys*, *Ehrlichia canis* and *Hepatozoon canis*), *Ixodes* spp. ticks (e.g. *Anaplasma phagocytophilum* and *Borrelia burgdorferi*) and mosquitoes (e.g. *Dirofilaria immitis*). In the Mediterranean basin, leishmaniosis due to *Leishmania infantum* is the most prevalent vector-borne disease in dogs. A broad range of clinical manifestations have been described in canine leishmaniosis. *Leishmania* infection in dogs may be manifested as a subclinical infection, a self-limiting disease, or a non-self-limiting and severe illness. Therefore, a clinical staging system in this infection is important to establish an accurate prognosis and treatment. However, host and parasite factors that determine clinical outcome are poorly understood. Dogs with clinical leishmaniosis are often concurrently infected with multiple pathogens, which are frequently vector-borne, such as *E. canis*, the causative agent for canine monocytic ehrlichiosis, *A. platys*, *Babesia vogeli* and *H. canis*, resulting in an unpredictable incubation period, atypical clinical outcome and poorer prognosis, compared with dogs infected with *L. infantum* alone. It has been reported that infections with other vector borne organisms can affect the severity of canine leishmaniosis or mimic its clinical signs and/or clinicopathological abnormalities. Several

studies have demonstrated that dogs exposed to numerous tick-borne co-infections have a higher relative risk of progression to clinical leishmaniosis. For this reason, veterinarians need to be suspicious of coinfections in dogs with clinical leishmaniosis when 1) presence of uncommon clinicopathological abnormalities; 2) presence of atypical clinical signs; 3) not responding well to treatment and 4) frequent clinical relapses. It is also important to highlight the use of specific treatment for each pathogen diagnosed. The prevention against ticks, fleas and sand flies is also extremely important. The aim of this talk is to provide an updated overview of co-infections with other vector-borne pathogens in dogs with leishmaniosis focusing on clinical aspects, diagnosis, prognosis, treatment and prevention.

Conflict of interest Laia Solano-Gallego and Gaetano Oliva do not have conflict of interest in this lecture.

S37-06: ASYMPTOMATIC DOG'S INFECTIOUSNESS: IS TREATMENT NECESSARY?

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Infected dogs are considered the main domestic animal reservoirs for *Leishmania infantum* parasite. Canine leishmaniasis (CanL) is a progressing disease characterized by a broad spectrum of signs, ranging from asymptomatic/subclinical infection, frequently characterized by negative *Leishmania* serology and general healthy status, to severe, fatal disease condition. Infectiousness of dogs to competent phlebotomine vectors has been associated with many factors, the main being the severity of the disease exhibited by infected dogs, with particular emphasis to the skin lesions. Many limitations in the definition of dog's infectiousness are due to a non-definitive proof of the role of subclinically infected dogs (i.e asymptomatic) in comparison with clinically ill animals in the contribution of Zoonotic Visceral Leishmaniasis (ZVL) transmission risk. Contradictory interpretation of results from different studies is likely to be due to the lack of standardized definition of subclinical/asymptomatic condition, as often it is referred to the only absence of overt clinical signs at the external inspection without considering the possible presence of clinicopathological alterations. Subclinical infection is featured by the detection of parasite by the PCR method and/or the appearance of low antibody titers. In these dogs, a strong cellular immune response persists, with a balanced levels of tissue-specific immunity to not cause immune-mediated tissue damage and subsequent disease. The severity of late-stage disease is correlated with high antibody levels and increasing parasite load. Sick dogs must be treated with anti-*Leishmania* specific drugs to decrease the parasite load that allows the clinical recovery. Xenodiagnosis studies indicated that anti-*Leishmania* drugs reduced or completely abolished canine infectiousness of sick dogs for at least 4 months. The combined use of second-line humans leishmanicidal

agents (e.g., pentavalent antimonials, miltefosine) and allopurinol is currently the first-line treatment for CanL, however some of these drugs have reported clinical resistance in dogs. Due to the lack of definitive confirmation of the infectiousness of subclinical infected dogs and because of their solid cellular immunity, it is recommended to avoid the treatment with specific anti-*Leishmania* drugs of these healthy dogs, to prevent possible side effects and drug-resistance too. What is important is the regular follow up of the infected healthy dogs, to treat them in case of the disease appearance, usually related to the increase of antibody titer.

The Author has not conflict of interest in this topic.

S38. THE CUTANEOUS LEISHMANIASIS IN THE MAGHREB REGION

S38-01: GENOMICS AND DATA SCIENCE APPLICATIONS FOR DIAGNOSIS OF CUTANEOUS LEISHMANIASES

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Due to their hyperendemicity, high morbidity and burden, and psycho-social impact, CL constitute major public health problems in North Africa where they list among top infectious diseases to control. In these countries, CL is caused by at least three species: *L. major*, *L. tropica* and *L. infantum*. Control programs are facing emergence and changing eco-epidemiological profiles mostly due to environmental and climate changes. Diagnosis of cases and species identification are central to treat and control these diseases. To address needs and gaps, we have engaged into the development of different solutions to support diagnosis, with national and international financial support while sustaining capacity building at the Institut Pasteur de Tunis with training of undergraduate and postgraduate students, Post docs and early career scientists. First, we have adopted different complementary strategies to deliver a range of DNA assays, based on different markers identified and validated by genome and phylogenetic analyses. These tests proved the principle of using them to support CL diagnosis, which then we sequentially engage into comparative evaluation on cutaneous samples. This includes a PCR HRM assay that identifies the different species encountered in MENA and Asia simultaneously to their detection; a multiplex DNA assay coupled to detection on dipstick; species specific isothermal RPA assays coupled to detection on dipsticks.

These projects notably with support from the PEER joint NAS-USAID program for the development of isothermal RPA assays allowed developing Leisionia, a digital system for the management and timely analysis of clinical, parasitological and epidemiological data. This software can be implemented in multicentric projects allowing dedicated and safe access to the data. Then we complemented this approach by implementing Artificial Intelligence algorithms to detect and classify skin lesions using simple photographs. These AI algorithms were integrated within a mobile version of Lesionia as a tool to guide CL diagnosis and patient management. We are currently planning to implement this application in controlled real-life conditions for CL diagnosis in a selection of hospital departments in Tunisia, before promoting its expanded use to more peripheral structures.

Our strategy currently fits the priorities defined by the 2030 NTD roadmap. We are currently engaged into the African Leishmaniasis consortium to promote the harmonized use of such technologies and tools for a better control of cutaneous leishmaniases in North, East and West Africa.

Current financial support: Ministry of Higher Education and Research – Tunisia, PEER- NAS-USAID program (PEER 518), Agence Universitaire de la Francophonie, African Academy of Sciences.

S38-02: CUTANEOUS LEISHMANIASIS IN THE MAGHREB: PUBLICATION METRICS AND COLLABORATION OPPORTUNITIES

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Morocco, Algeria and Tunisia are located in North-western Africa and are commonly known as the Maghreb region. Due to geographical, climatic, socio-economic and cultural similarities, it could be possible to find numerous identical control management strategies against some diseases that threaten the population of these countries.

During these ten years, some scientific publications targeted the Leishmaniasis disease in the Maghreb. However, PubMed metrics of that publications show some differences. For example, epidemiological studies reported the existence of both visceral and localised cutaneous leishmaniasis (CL) forms. A difference in transmission ways for anthroponotic CL due to *L tropica (Killicki)* compared to the zoonotic CL due to *L. major* or *L.infantum*. The latest is causative for cutaneous and visceral forms. The vectors are also well defined, while more host reservoirs are subject to confirmation, especially for the anthroponotic form that could be sustained due to some non-human reservoir pools of CL. However, during the last ten years, leishmaniasis publications in the Maghreb countries have remained under 2%. (225/14993) (compared to the overall journal publications about the leishmaniasis-affected areas in the world). Compared to other endemic areas in the Eastern Mediterranean Region, namely Saudi Arabia, Egypt and Pakistan, English is considered the first foreign language. At the same time, French is the first language in the Maghreb. It seems that publications in the English language had the same growth over the past two decades, and the potential of English publications has become more important in Morocco compared to Tunisia and Algeria. It should be reminded that until 2017 Moroccan authors were systematically eligible to fully waive processing charges in many peer-reviewed journals,

thanks to the Research4Life initiative supported mainly by WHO / World Bank and other similar initiatives. The absence of financial barriers to publication could partly explain this increased number of publications. While today, all three countries are listed in Group B, allowing only low-cost access that will impact the trend soon. Moreover, the articles published by only researchers from the same country without any international co-authorship collaboration reached 39% in Algeria, 53% in Tunisia and 61% in Morocco from all the published articles in English, including at least one researcher as co-author during the last ten years. That could be explained firstly by the ease of publishing free of charge without the need for financial support in some journals interested in the Neglected tropical diseases research or supporting the list of eligible countries for waiver fees. Secondly, the decision to publish by some Moroccan researchers in peer-reviewed non-open access journals messes the opportunity to be worldwide read and shared in free, easy-to-use copyright Creative Commons licenses.

Focusing on Morocco, the international collaboration helped provide evidence about CL vector control, host reservoirs & species mapping, and diagnostic tools accuracies. Understanding the preventive ways for zoonotic and anthroponotic CL and the psycho-social burden linked to this disease in endemic areas and documenting the field control management.

Each of the three countries has an important number of international bilateral collaborations, mainly with European countries. With few collaborations, projects between Tunisia and Algeria or between Morocco and Tunisia are specifically conceptualised within the Pasteur Institute network. Unfortunately, no collaboration has existed between the three countries during the last ten years. Answering sometimes the same research questions but in different areas and timing. They lead to weak scientific evidence, while concomitant multicentric studies in the Maghreb will be more scientifically interesting.

The two main examples, first, are the assessment of the accuracy of some rapid diagnostic tests for CL without reaching all CL species variations that exist in the Maghreb and the presentation of a few numbers of participants from each country with an impact on the results. The second example is the

control of the Zoonotic CL in the country's border areas, decreasing the effectiveness of the field intervention targeting rodents by the poisoned wheat baits without any possibility to start synchronous treatment on both sides of the borders.

Such collaboration could positively influence the yearly ZCL human case numbers due to *L. major*, which seems to follow a cyclicity of 5 to 10 years of outbreaks in which only simultaneous treatment activity targeting rodent populations or vector density will succeed. At the same time, the ACL remains in Morocco with the same yearly cases even during COVID-19 restrictions and acquired herd immunity vaccination, which invites more exploration of any new or forgotten animal or host reservoir for *L. tropica* and its complex immunopathology. Many interesting articles are available from each country of the Maghreb, and there is a need to complete the evidence of each country's research question from all Maghreb. In addition, the complexity of the CL burden should have more proof within each county's context.

Finally, is it the right time to build the "Maghreb Leish" initiative? That could be the process of introducing well-designed clinical trial multicentric studies. We can wonder about making a Big Maghreb collaboration, including Mauritania and Libya, to generate the best scientific evidence that could be valuable and comparable to other countries' Leishmaniasis work groups.

S39. DRUG RESISTANCE & QUIESCENCE: UNRAVELLING MECHANISMS AND EXPLOITATION FOR BETTER/NEW DRUGS

S39-01: GENOMIC SCREENS FOR DRUG RESISTANCE STUDIES IN *Leishmania*

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Studies of drug resistance in *Leishmania* can help in providing tools for detecting resistance in the field but can also lead to drug targets and a better understanding of drug's mode of action. We developed a number of genome wide screens exploiting drug resistance coupled to next-generation sequencing (NGS). Selecting parasites for resistance and sequencing the resistant organisms (Sel-seq) can lead to loss- and gain of function mutations. This stepwise selection is time consuming, however, and chemical mutagenesis can expedite the generation of resistant parasites that are characterized by NGS (Mut-seq) also leading to both loss- and gain of function mutations. Transformation of cosmid libraries in *Leishmania* and selection for increased resistance lead to enrichment of cosmids that are characterized by NGS (Cos-seq) and that lead to gain of function mutations. Finally, we developed a CRISPR-Cas9 whole genome screen where enriched guides following drug selection are subjected to NGS and lead to loss of function mutations. Those four screens were used against all currently approved anti-*Leishmania* drugs but also against a plethora of experimental active compounds against *Leishmania*. We will present an overview of our most recent significant findings while discussing strengths and weaknesses of each screens.

S39-02: UNRAVELING QUIESCENT PHENOTYPES AS AN ADAPTATION TO OVERCOME DRUG PRESSURE IN *Leishmania*

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Resistance against the currently available Leishmanicidal drugs has been studied for years, giving important insights into how *Leishmania* adapt to these drugs and select heritable traits that make a new parasite population unsusceptible to these drugs. However, *Leishmania* can also survive lethal drug exposure without heritable genetic resistance, a rather unexplored phenomenon. We show here that under antimonial drug pressure *Leishmania* can adopt two survival phenotypes associated with a quiescent state: i) tolerant cells, which constitutes a considerable proportion of the population, and ii) persisters a phenotype acquired by a very small fraction of the population. We studied a panel of 9 isogenic *Leishmania braziliensis* lines highly susceptible to potassium antimonyl tartrate (PAT, median IC50 of promastigotes = 2.17 µ/mL, IQR= 2.88). We exposed promastigotes to lethal PAT pressure (4 times the median IC_{50} for 48 hrs), transferred surviving cells to fresh medium without drug pressure and showed that these parasites resumed proliferation and expanded to a population that retained the original susceptibility to PAT. Under PAT drug pressure, the 9 lines showed evidence of arrested growth and a variable proportion of cellular death. The quantification of their rEGFP expression (a biosensor of quiescence) indicated that under drug pressure, all lines had in average a 30 % reduction in their biosynthetic metabolism compared to proliferating cells and, more importantly, a dose-response relationship between the

downregulation of rEGFP and increasing concentration of drug. The exploration of persisters was also undertaken in one of the isogenic lines by longer exposure to the drug. After 14 days of exposure to PAT (~ 4 fold the IC_{50}), less than 1 % of the population survived, as indicated by flow cytometry and confocal microscopy. This subpopulation had a deeper downregulation of their rEGFP expression (~ 85 % reduction compared to proliferating cells) and increased tolerance to a second PAT exposure. We also showed that quiescent cells that arise from stationary phase starvation (~ 10 % of the population) can also survive the same time frame as persisters. However, they die rapidly if faced with PAT pressure as they do not have the increased drug tolerance observed in persisters. Our results suggest quiescent cells emerge rapidly in response to PAT pressure. Moreover, quiescence may encompass non-proliferative cells with different physiological adaptations, depending on the environment that triggered it. We have unraveled new phenotypic diversity deployed by *Leishmania* to overcome drug pressure and discuss how it may hamper the efficacy of currently available drugs.

S39-03: TACKLING PERSISTERS IN CHAGAS DISEASE DRUG DISCOVERY.

Manu De Rycker on behalf of the DDU/GSK Kinetoplastid Drug Discovery Team[1,2]

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Chagas disease, caused by the protozoan intracellular parasite *Trypanosoma cruzi*, is a highly neglected tropical disease, causing significant morbidity and mortality in Central and South America. It affects approximately 6-7 million people worldwide, with an estimated 1.2 million suffering from Chagasic cardiopathy and results in more than 10,000 deaths annually. Currently, the nitroaromatic compounds benznidazole and nifurtimox are the only drugs approved for treatment of Chagas disease. Both drugs cause important side-effects and are not consistently efficacious for the treatment of chronic Chagas disease, the most prevalent clinical presentation. The lack of new drugs for this disease reflects the many challenges associated with Chagas drug discovery, such as wide tissue distribution of the parasites, lack of good clinical markers for cure and a relatively limited amount of funding for drug discovery. To add to these challenges, recent data indicates that a small subpopulation of *T. cruzi* parasites is less susceptible to treatment. Our work demonstrates the challenges that such persisters pose for Chagas disease drug discovery, but also reveals opportunities to tackle this problem. The persister assays that we have developed allow us to predict if compounds will be able to clear all parasites in animal models. While few mechanisms of action appear to eliminate all parasites in monotherapy, we show that combination therapy is a promising approach to overcome this problem.

S39-04: A STRUCTURE-BASED DRUG DISCOVERY PROGRAM TARGETING *LEISHMANIA* GLYCOGEN SYNTHASE KINASE 3

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Leishmaniasis is a complex vector-borne disease caused by the protozoan parasite *Leishmania spp*. Infection with parasites results in diverse clinical manifestations, ranging from localized skin ulcers in cutaneous leishmaniasis to potentially fatal systemic disease in visceral leishmaniasis. The disease has strong links with poverty, with over a million estimated new cases per year worldwide. Despite its large health and social impact, leishmaniasis is not seen as a priority by the pharmaceutical community and policymakers, it thereby remains a truly neglected tropical disease. Drugs currently available are toxic, antiquated, not adapted to the field, and show variable efficacy, there is, therefore, a clear need for new treatment options. Phenotypic-based drug discovery campaigns allowed an unprecedented portfolio of new chemical agents to recently progress to clinical evaluation. However, the development of new drugs to treat leishmaniasis has been hampered by the difficulty of identifying valid therapeutic targets. Protein kinases are promising candidates for drug development against parasitic diseases. The long and short isoforms of *Leishmania* glycogen synthase kinase 3 (GSK3a/b, respectively), a multifunctional Ser/Thr kinase, have been identified as genetically essential for parasite viability and have been pharmacologically explored to some extent. In this work the team reports

efforts within a Latin American Open-Science target-based drug discovery program to develop potent and selective inhibitors of *Leishmania infantum* GSK3a and GSK3b. Recombinant protein kinases were produced and screened against a compound library obtained from commercial sources. Biochemical assays were employed to validate positive hits and to determine IC_{50} and Ki values. Promising compounds (active in the nanomolar range) were further investigated by cellular assays to evaluate antileishmanial activity and host cell toxicity. Additional data was generated to support the prioritization of chemical scaffolds, such as selectivity over a panel of human kinases and ADME properties. The team is currently analyzing data obtained from protein-inhibitor complexes to elucidate ligand-binding modes and to guide the design of optimized analogues for two different chemical series. The goal of the current project stage is to enable the progression of lead compounds to proof-of-concept studies in animal models of the disease.

Keywords *Leishmania*; DRUG DISCOVERY; DRUG TARGET; GSK3.

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S40. IMMUNOLOGICAL PERSPECTIVES OF LEISHMANIASIS: BEYOND THE TH1/TH2 PARADIGM

S40-01: TISSUE- AND DRUG-SPECIFIC TRANSCRIPTOME DYNAMICS OF CD4$^+$ T CELLS DURING EXPERIMENTAL VISCERAL LEISHMANIASIS

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We previously generated transcriptional profiles of peripheral blood CD4$^+$ T cells from visceral leishmaniasis (VL) patients and identified type I IFN pathways as major upstream regulators of anti-parasitic CD4$^+$ T cell responses. We also assessed the transcriptional profiles of CD4$^+$ T cells from VL patients before and after drug (AmBisome) treatment and by comparing with tissue-specific CD4$^+$ T cell transcriptional profiles from experimental VL, we identified the NK cell granule protein NKG7 as a novel regulator of inflammation in a broad range of diseases. These data sets generated new knowledge and provided key insights into disease pathogenesis. However, information from bulk RNA sequencing of CD4$^+$ T cells is constrained by an inability to determine the contributions of different cell subsets to transcriptional outputs, establish CD4$^+$ T cell subset hierarchies and their relationships, as well as identify cells transitioning between states. Such information is important if trying to modify host responses for clinical advantage because such knowledge can help avoid unwanted consequences of such therapies. A powerful approach to overcome these limitations is single cell RNA sequencing (scRNAseq). This method also allows us to re-evaluate CD4$^+$ T cell responses in specific tissue and disease settings taking into account relationships between CD4$^+$ T cell subsets and their functional

potential over the course of infection. We have performed a scRNAseq experiment in experimental VL using *Leishmania*-specific $CD4^+$ T cell receptor (TCR) transgenic cells (PEPCK) to investigate tissue- and drug-specific responses. We also employed a *Leishmania*-specific MHC-class II I-A^b tetramer ($PEPCK_{335-351}$) to examine the entire complexity of the $CD4^+$ T cell response encompassing a spectrum of TCR specificities. Results from the analysis of this data set will be presented.

S40-02: ORAL TOLERANCE INDUCED BY HEAT SHOCK PROTEIN 65-PRODUCING *Lactococcus lactis* MITIGATES INFLAMMATION IN *Leishmania braziliensis* INFECTION

Priscila Guerra[1], Camila Andrade[1], Ivanéia Nunes[1], Brena Gama[1], Rafael Tibúrcio[1], Washington Luis Conrado Santos[1,2], Natalia Machado Tavares[1,3], Juliana Rebouças[6], Tatiani Maiolii[4], Ana Caetano Faria[3,5], Cláudia Ida Brodskyn[1,3]

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Cutaneous leishmaniasis caused by *Leishmania braziliensis* induces a pronounced Th1 inflammatory response characterized by IFN-γ production. Even in the absence of parasites, lesions result from a severe inflammatory response in which inflammatory cytokines play an important role. Different approaches have been used to evaluate the therapeutic potential of orally administrated heat shock proteins (Hsp). These proteins are evolutionarily preserved from bacteria to humans, highly expressed under inflammatory conditions and described as immunodominant antigens. Tolerance induced by the oral administration of Hsp65 is capable of suppressing inflammation and inducing differentiation in regulatory cells, and has been successfully demonstrated in several experimental models of autoimmune and inflammatory diseases. We initially administered recombinant *Lactococcus lactis (L. lactis)* prior to infection as a proof of concept, in order to verify its immunomodulatory potential in the inflammatory response arising from *L.*

braziliensis. Using this experimental approach, we demonstrated that the oral administration of a recombinant *L. lactis* strain, which produces and secretes Hsp65 from *Mycobacterium leprae* directly into the gut, mitigated the effects of inflammation caused by *L. braziliensis* infection in association or not with PAM 3CSK4 **(***N*-α-Palmitoyl-*S*-[2,3-bis(palmitoyloxy)-(2*RS*)-propyl]-L-cysteine, a TLR2 agonist). This was evidenced by the production of anti-inflammatory cytokines and the expansion of regulatory T cells in the draining lymph nodes of BALB/c mice. Our *in vitro* experimental results suggest that IL-10, TLR-2 and LAP are important immunomodulators in *L. braziliensis* infection. In addition, recombinant *L. lactis* administered 4 weeks after infection was observed to decrease lesion size, as well as the number of parasites, and produced a higher IL-10 production and decrease IFN-γ secretion. Together, these results indicate that Hsp65-producing *L. lactis* can be considered as an alternative candidate for treatment in both autoimmune diseases, as well as in chronic infections that cause inflammatory disease.

Keywords *Lactococcus lactis;* Hsp65; *Leishmania braziliensis;* IMMUNOREGULATION

Financing Conselho Nacional de Pesquisa (CNPq) and Coordenação de Aperfeiçoamento de Nivel Superior (CAPES)

S40-03: CD4 T CELL MAINTENANCE IN A CHRONIC INFLAMMATORY ENVIRONMENT

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Maintenance of functional CD4 T cell responses is crucial for controlling *Leishmania donovani* growth during persistent infection. However, protective Th1 responses straggle to fully develop in the hostile inflammatory environment that characterizes the spleen during chronic VL. Indeed, Th1 cells are not only suppressed by Tr1 cells and MDSCs (myeloid-derived suppressor cells), but they are also showing signs of functional exhaustion as well as dying following sensing of tissue damage-derived apoptotic cell material via TLR7. Here we discuss a novel CD4 T cell subset that arise during chronic experimental VL and can give rise to Th1 and Tr1 cells. This subset displays a unique gene signature that relates to that of Th1 memory and hematopoietic stem cell progenitors, but is distinct from that of Th1 cells. These cells are antigen-specific and can differentiate into Th1 and Tr1 responses upon transfer to a *Rag1*$^{-/-}$ mouse after infection with *L. donovani.* Our findings suggest that this unique subset may be responsible for replenishing functionally exhausted and/or dying CD4 T cells during persistent *L. donovani* infection in mice.

S41. WHAT CAN SOCIAL SCIENCES CONTRIBUTE TO UNDERSTANDING AND ADDRESSING LEISHMANIASIS?: EXAMPLES FROM THE FIELD

S41-01: WHAT CAN SOCIAL SCIENCES CONTRIBUTE TO UNDERSTANDING AND ADDRESSING LEISHMANIASIS?: EXAMPLES FROM THE FIELD

Mady Barbeitas[1], Lina Pinto-García[2]

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In the mid-2000s, the World Health Organization (WHO) began developing institutional actions to address the social determinants of health at the global, national, and local levels. This could be read as an attempt to return to the Declaration of Alma-Ata by creating a new strategy to commit countries to reactivate Primary Health Care activities. Such determinants consist of the conditions in which people are born, grow, work, live, and age, as well as the broader set of forces and systems shaping health and disease in daily life. This "new" framework has come to occupy a prominent place in global health, most recently with the creation of a new Department of Social Determinants of Health in the context of the WHO General Programme of Work (2019-2023). "Why treat people's illnesses without changing what made them sick in the first place?" is one of the WHO statements that have summarized what this approach is all abouthttps://www.zotero.org/google-docs/?OYIKg0. Despite these efforts and the urgent need to draw attention to and address the persistent political, economic, cultural, and socio-environmental factors that maintain inequities, vulnerability, susceptibility,

and high risk for neglected tropical diseases such as leishmaniasis, the dominant strategies in both biomedical research and public health programs still focus on pharmaceuticals. In dialogue with the social determinants frameworkhttps://www.zotero.org/google-docs/?UtEeAn, social scientists from fields as varied as Critical Medical Anthropology, Social Studies of Science & Technology (STS), Sociology of Health & Illness, History of Medicine, and Health Humanities have insisted, based on a large body of theoretical elaborations and empirical findings, on the need to understand and address first and foremost the root causes of poor health. However, there are still very few genuinely interdisciplinary initiatives in which biomedical scientists and epidemiologists collaborate horizontally with anthropologists, sociologists, and historians to make visible and challenge the root causes of disease and health inequities. In fact, health sciences tend to invoke the participation of social scientists only when it is deemed necessary that a technology or an intervention has better acceptance by a particular community, or when non-biomedical knowledge and treatments are thought to pose a threat to the biomedical management of a particular health problem. However, this view of what social science does, contributes, and brings to the table is quite limited. This WorldLeish7 panel aims to bring together a range of scholarly efforts that exemplify the work of academics exploring the social life of leishmaniasis in different contexts across the world. Some of the leishmaniases-related topics we want to address in this panel, from a social science theoretical and empirical perspective, are:

- Root causes of cutaneous and visceral leishmaniasis
- Illness experience, stigma, and meaning-making
- Structural barriers to leishmaniasis diagnosis and treatment
- Patient journey and help-seeking behaviors
- Biomedical research and the contexts in which leishmaniasis scientists work
- Clinical trials and studies
- The politics of innovation
- Inclusion and exclusion in public health regulations and dispositions
- The social life of antileishmanial therapies
- Access to pharmaceuticals

S41-02: HOW NEGLECTED DISEASES CAN SHAPE PHARMACEUTICAL MARKETS? LOCAL AND GLOBAL POLITICS OF INNOVATION FOR LEISHMANIASIS

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Previously framed as tropical diseases, cutaneous and visceral leishmaniasis were classified as neglected tropical diseases (NTD) in 2005 by the World Health Organisation. However, since the 1990s the term "neglected" has been used to capture funds and political attention for a group of parasitic and vector-borne diseases which lacks of adapted treatments. In the beginning, this term denounced the lack of R&D investments from pharma industries to bring new treatments for the so-called 'insolvent markets' (market failure concept). In fact, 'insolvent market' was the main feature to gather these diseases. The growing importance of NTDs in the global political arena has mobilized several actors, including public research institutes and international organizations. They have approached the private sector to share the costs and risks of developing new drugs for leishmaniasis, establishing public-private partnerships (PPPs). Drawing on the fields of critical Global Health and Social Studies of Science and Technology, this work is based on ethnographic research at some PPPs created to develop new drugs for leishmaniasis. It explores drug candidates' trajectories to review the multiple starts, deviations, pauses and restarts in order to appreciate the complexity of actors, stakeholders, financial challenges and power relations at work in drug-development PPPs. By studying drug trajectories, this research proposes a detailed panorama of the "lives" of leishmaniasis drug candidates through the lens of actors and stakeholders involved in this process.

S41-03: BACK TO DUTY RATHER THAN BACK TO HEALTH: LEISHMANIASIS AND THE COLOMBIAN ARMY

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Painless skin sores, which grow slowly and resist healing, are the only physical manifestation of cutaneous leishmaniasis. These lesions appear when the mantablanca—a tiny sandfly that is sometimes infected with microscopic *Leishmania* parasites—encounters a human source of blood in the jungle and bites. Although leishmaniasis has historically affected all sorts of people whose daily lives are entangled in one way or another with the Colombian jungle, its prevalence among servicemen has been particularly high. In fact, leishmaniasis is inherent to soldiering in Colombia, part of the vicissitudes of the military role. Drawing on the fields of Critical Medical Anthropology and Social Studies of Science and Technology, this work is based on ethnographic research at the military Leishmaniasis Recovery Center (CRL). It explores the context in which this disease became a strategic problem for the Army in the state war against guerrillas. I pay attention to the drastic measures adopted by this institution when it became clear that maintaining the state military force largely depended on healing leishmaniasis lesions to restore the fighting capacity of soldiers effectively. I argue that, in the mid-2000s, leishmaniasis rehabilitation became integral to military medicine for its ability to bring soldiers back to duty rather than back to health. Thus, keeping manpower resources available for the war turned into the military mission assigned to medical and military personnel in charge of treating leishmaniasis within the Army. Also, since that moment, the massive use of antileishmanial drugs became crucial to maximize the extraction of residual labor available for war in the body of each soldier

affected by leishmaniasis. After being pharmaceutically recycled, again and again, the soldier's body wears out slowly until becoming disposable. Through that violent process, war does not remain confined to the jungle, but overflows this emblematic space of the Colombian armed conflict with the crucial participation of biomedical knowledge, pharmaceuticals, and healthcare practices.

S41-04: HISTORY OF THE NEW WORLD LEISHMANIASES: AN OVERVIEW

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"Leishmaniasis is a disease of dogs and those who lead a dog's life," wrote a parasitologist from the Brazilian state of Ceará (Alencar, 1959). That's still true, but in Brazil the disease is breaking the social and geographical barriers that characterized its original distribution. In post-war publications by Brazilian researchers, one observes growing dialogue and cooperation with American, European and Asian workers. International collaborations are influenced by programs and initiatives of WHO, PAHO and other organizations, and by the fact that leishmaniases resurge in rural areas where they had apparently been eliminated, and they spread in peri-urban areas, cities and regions considered free of the endemic. The global process of increasing morbidity and mortality is ascribed to climate and ecological changes that affect the distribution of vectors, to population displacement, and to expanding economic flow and chaotic urbanization processes. Larger population groups and new social groups are being infected. Leishmaniases presents another troubling aspect. The toxicity, unreliability and high cost of the therapeutics used in their treatment. Although leishmaniasis represents a great a risk to human health, it continues to be more or less unimportant on public health agendas. In reality, a growing contrast between neglect and invisibility of leishmaniasis in public health policies and increased interest in scientific research on the subject can be observed. Specialists in new or traditional institutions are turning to this group of diseases and identifying new outbreaks in Latin America, Asia, Africa and even Europe. A good way to initiate the study of their trajectories and of the network to which they are connected is to address the relevant contributions of British scientists to research on leishmaniases done in Brazil. In 1965 at the Instituto Evandro Chagas, Ralph Lainson (recently deceased) and Jeffrey Shaw founded the Wellcome Parasitology Unit. There

they made important contributions to the study of leishmaniases and became globally recognized authorities on the taxonomy of the protozoa connected to these diseases. Philip Marsden began his work in Brazil in 1967, as a professor of tropical medicine at the University of Brasilia. There he became a leading authority in control of leishmaniases until his death, in 1997. Toby Barrett worked at the Instituto Nacional de Pesquisas da Amazônia- INPA (National Institute of Amazonian Research) in Manaus, where he dedicated his life to field studies on this group of diseases. At the same time, investigations on leishmaniasis were reframed by molecular biology. It is important to evaluate historically the impact that this discipline had on the production of new knowledge about all aspects of the leishmaniasis, including the development of therapeutics and vaccines. The introduction of molecular biology at Fiocruz took place in a climate of major restructuring of the institution as well as of Brazilian health policies by force of a health reform movement and of Brazil's redemocratization process. Fiocruz became one of the pillars of Brazil's Unified Health System (SUS), that materialized at the legal-political level (but not yet at the practical level) the most daring aspirations proposed by the Alma-Ata Declaration (1978). The blossoming of research at Fiocruz was due to a return to old traditions and to interactions between experienced scientists and new generations of workers coming from the graduate programs that greatly expanded during Brazil's military dictatorship and after its return to democracy.

S41-05: THE ORIENTALIST'S SORE: BIOMEDICAL DISCOURSES, CAPITAL AND URBAN WARFARE IN THE COLONIAL PRESENT

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This presentation examines the processes leading to the emergence of similar cutaneous leishmaniasis epidemics in two very different populations, citizens of Ouagadougou and U.S. soldiers deployed to Iraq, and the diverging responses of biomedical authorities to these two outbreaks. Based on fieldwork in Burkina Faso and on scholarly research, il frames the discussion in spatial terms examining the intersecting pathways of the "soft" logic of capital accumulation and the "hard" logic of warfare which both target and shape the urban landscape. However, the ascendency of these logics becomes mystified by a microbiological imaginary which concomitantly orientalizes the urban spaces and diseases of a purported Other, yet perceives the military challenges posed by urban insurgencies and by cutaneous leishmaniasis as particular logistical pathologies. To do so, these discursive regimes employ powerful imaginative geographies that establish differences between us and them, and between here and there, in order to blur the ties between the two logics of capital and warfare. Essential to the upholding of this mystification is the ongoing construction of Orientalist discourses relying on the insights and tools of the germ theory of disease which span the work of Pasteurians and extend to the colonial present.

Keywords CUTANEOUS LEISHMANISIASIS; IMAGINATIVE GEOGRAPHIES; OUAGAGOUDOU; OPERATION IRAQI FREEDOM

S42. MUCOCUTANEOUS LEISHMANIASIS

S42-01: HOW TO REACH THE PARASITOLOGICAL DIAGNOSIS IN ML?

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ML is associated with multiple factors, such as the parasite species, immune response, and the viral endosymbiont *Leishmania* RNA Virus 1 (LRV1). Being a chronic parasitological disease, laboratory diagnosis of ML poses a challenge for health services. Our group has studied cases of mucosal leishmaniasis in patients treated at the Reference Center in Rondônia, in the north region of Brazil. This region constitutes the most significant cases of tegumentary leishmaniasis (TL) in the country and is the region with the highest diversity of species of vectors and *Leishmania* parasites ever described. In this region, mucosal leishmaniasis (ML), a clinical form of TL, exceeds the national average of cases, reaching up to 12% of the total annual TL notifications. We evaluated more than 700 clinical samples from patients with clinical suspicion of TL, including patients with cutaneous (CL) and mucosal leishmaniasis, comparing the results of parasitological tests: direct parasitological examination by microscopy (DP) and conventional PCR (cPCR) targeting both kDNA and hsp70. DP was performed by collecting material from lesions through biopsies (mucosal lesions) or scarification (cutaneous lesions); for PCR, a cervical brush was used for sample collection. Blood samples were tested employing standardized Real-Time

PCR (qPCR) protocol targeting the HSP70 gene. PCR tests showed higher sensitivity than DP for both CL and ML samples. Considering ML samples only (N = 89), DP showed a sensitivity of 49.4% (N = 44) against 98.8% (N = 88) for kDNA PCR. The qPCR hsp70 for blood samples from patients with ML (N = 14) resulted in superior sensitivity (50%; N = 7) compared to DP (21.4%; N= 3) for samples from the same patients. Our results reinforced the need to implement a molecular test for the diagnosis of ML, in addition to proposing methods less invasive for collecting material from TL patients. Sample collection using cervical brush in lesions observed in CL and ML patients is easy to perform and less invasive, compared to scarification and biopsies. Blood samples could be a good source for qPCR diagnosis for ML patients. Thus, we propose here a standardized method for collection and performing molecular diagnosis of clinical samples from suspicious ML patients that can be applied in reference services for improving ML diagnosis.

S42-02: EXPERIENCE IN THE TREATMENT OF MUCOSAL LEISHMANIASIS IN MILITARY PERSONNEL IN COLOMBIA

Claudia Marcela Cruz Carranza

Number Member ASOCOLDERMA

Members of the Colombian Army are prone to vector-borne diseases due to the displacement they make during military operations through various endemic areas of the country. In the last five years, 8274 cases of Leishmaniasis were reported, of which 8195 cases correspond to L. cutaneous and 79 cases to L. mucosa. A retrospective descriptive analysis of the cases of mucosal leishmaniasis reported by the Colombian Army to the National Public Health Surveillance System from 2017 to 2021 is carried out. We analyzed 79 cases of L. Mucosa finding that all cases correspond to men aged between 18 and 67 years; 71% of the cases are concentrated in the departments of Meta (25%), Guaviare (22%), Nariño (10%), Guainía (8%) and Antioquia (6%). The time elapsed from the onset of symptoms to the consultation ranges from 5 days to 187 days. Patients presented with mucosal ulcer, rhinorrhea, epistaxis, nasal obstruction and hyperemia. The most affected mucous membranes were the nasal, oral cavity and lips (89%). HIV co-infection is unknown in 63% of cases and did not coexist in 37%. Pharmacological treatment was performed for 92% of patients with meglumine antimony and the remaining 8% with pentamidine isethionate, which meant a cost of COP $39,417,446.5 during this five-year period; equivalent to COP $527,200 per patient treated with antimonial and COP $243,176 per patient treated with pentamidine. 30% of patients had previously undergone treatment for Leishmaniasis. Thecondition of the nasal mucosa and the clinical findings of our patients correspond to what is described in the literature. Pentavalent antimonials and pentamidine isethionate were used according to the recommendations of the treatment guidelines. With the notification form to the National Public Health Surveillance System, it is not possible to determine adverse effects to the drugs, cure rates or therapeutic failure and if referral to another level of

complexity is required to establish another line of treatment until the cure criteria are met. The most frequently identified adverse events when administering pentavalent antimonials are musculoskeletal pain, gastrointestinal problems, headache, electrocardiographic changes, and increased liver and pancreatic enzymes. With pentamidine isotianate, pain and edema at the site of application, abscesses, dizziness, fever, headache, adinamia, nausea and joint pain, acute hypotension, hypoglycemia and prolongation of QT may occur. It is necessary to have other treatment options with fewer adverse effects, cost andfectivos, easy administration and greater adherence.

S42-03: ML COSTS AND THEIR IMPACT ON PROGRAM COVERAGE

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Mucosal leishmaniasis (ML) is a severe form of tegumentary leishmaniasis (TL) and, generally, a late complication of the *Leishmania (Viannia) braziliensis* infection that usually occurs years after the cutaneous form. One factor that can increase morbidity is the small therapeutic arsenal available for ML, which is administered exclusively parenterally and has high toxicity.

ML poses a serious public health problem, and its progressive and destructive nature leads to extensive morbidity. We lack adequate research in ML and public policies aimed at coping with it. Similar to the trials addressing efficacy and safety, there are limited partial (cost estimation) or total (cost-effectiveness analysis) economic studies focusing on ML. Cost estimation is one of the first steps in economic assessment that calculates the costs involved in a given health intervention. In Brazil, we have performed an estimate of the direct medical costs of the therapeutic options for ML—meglumine antimoniate and liposomal amphotericin B—currently recommended as the first-line treatment in Brazil in addition to miltefosine. This would be the first step toward establishing a cost-effective treatment for ML. In addition, a critical analysis of the implications of costs on the performance of public programs in the countries of the Americas in the light of the new therapeutic recommendations produced by PAHO will be done.

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S42-04: LONG-TERM FOLLOW UP IN MUCOSAL LEISHMANIASIS

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Mucosal leishmaniasis (ML) is a difficult-to-manage disease with an uncertain therapeutic response, especially when the patient consults late and lesions are advanced. The recommendation in most national guidelines is to follow patients for one to two years after treatment, but most patients do not return to controls, so the determination of cure rates is based on very short follow-up of a reduced number of patients. This means that we may have false or, at least, incomplete information about the reality of the therapeutic effect of the drugs currently in use. At the Jorochito Dermatological Hospital (JDH) we analyzed what happened to the cutaneous and mucosal patients treated between 1999 and 2020. As the majority did not attend or attended very few follow-ups, we organized an active search for patients and here we present the results of ML we could find. In total, 475 subjects with a proven diagnosis of LM were treated, of which 355 were treated by the first time while 120 were recurrences of treatments received in other institutions.

Table 1 details the treatments received for cutaneous leishmaniasis (CL) that the patients had months or years before developing the mucosal lesion and the result depending on whether the treatment was well done or poorly done. 80% received parenteral pentavalent antimonials (PA) and when used correctly 85% of patients cured, while when lower doses or shorter times were used or pauses were made during treatment, the cure rate was reduced to 60%.

Table 1.	Done n (%)	well done n (%)	Cure n (%)	incorrectly done* n (%)	Result in Cure n (%)
Glucantime IM	287 (60.4)	241 (84)	207 (85.9)	46 (16)	30 (65.2)
Glucantime IV	93 (19.6)	88 (94.6)	80 (90.9)	5 (5.4)	3 (60)
Amphot B Deoxi	55 (11.6)	30 (54.5)	23 (76.7)	25 (45.5)	12 (48)
Miltefosine	19 (4.0)	17 (89.5)	15 (88.2)	2 (10.5)	1 (59)
unknown	21 (44)				

Treatment at JDH for naïve patients is presented in Table 2.

Table 2.	Treated (n=355) n (%)	Improved at the end of therapy n (%)	Cured at 6 to 12 mo follow up Cured/ Attended (%)
Glucantime IM	78 (22)	55 (70.5)	13/20 (65)
Glucantime IV	161 (45.4)	132 (82)	30/39 (76.9)
Amphot B Deoxi	50 (14.1)	33 (66)	12/19 (63.1
Miltefosine	66 (18.6)	52 (79)	28/36 (77.8)

Only 32% of treated patients attended controls for 6 or 12 months. The first treatment administered in JDH to the 120 patients with relapses of LM is detailed in Table 3.

Table 3.	Treated (n=120) n (%)	Improved at the end of therapy n (%)	Cured at 6 to 12 mo follow up Cured/attended (%)
Glucantime IM	8 (6.7)	5 (62.5)	2/5 (40)
Glucantime IV	33 (27.5)	22 (66.7)	8/17 (47.1)
Amphot. B deoxi.	16 (13.3)	9 (56.3)	3/7 (42.9)
AmphB liposom.	6 (5)	4 (66.7)	4/5 (80)
Miltefosine	23 (19.2)	15 (65.2)	9/15 (60)
Gluc IV + Milte	16 (13.3)	13 (81.3)	6/9 (66.6)
AmpB deox + Milte	11 (9.2)	8 (72.7)	5/8 (62.5)
Gluc IV + Pentoxyphilline	7 (5.8)	4 (57.1)	2/4 (50)

58% of the treated patients attended controls from 6 to 12 months, which reflects a greater degree of interest given that they had already been treated and had failed and because, over time, the lesions progress and the symptoms are more severe.

Since most of the patients did not attend the follow-ups and, therefore, there was no information on the result of the treatment, we organized an active search for these patients.

During Active Search we look for 324 out of original 355 naive ML patients (eliminating those 31 with therapeutic failure diagnosed during the first year of follow up in Jorochito). We found 142 patients and 63 of them (44.4%) had relapsed at least once.

Table 4: Failure rate in each treatment group of Active Search patients

Drug	Contacted	Failed	%
Glucantime IM	28	14	50
Glucantime IV	65	29	44.6
Amphot deoxi	16	9	56.3
Miltefosine	33	11	33.3

Table 5: Time to relapse in 63patients who failed (in years)

1	7
2	11
3 to 5	35
6 to 10	6
more than 10	4

If the one-year follow-up recommendation is followed, 90% of relapses will not be detected, while if it is extended to two years, 83% will not. According to these data, the ideal would be to follow the patients for at least five years in order to detect the majority of treatment failures.

Table 6 shows the times these 63 patients presented relapses.

1	22
2	15
3	11
4	9
5 or more	6

If we calculate the proportion of treatment failures in patients with a first episode of LM based on the data of the patients who attended controls from 6 to 12 months at the JDH, we would have a figure of 29.3%, while when the active search is done the percentage of failures rises to 44.4%. Unfortunately, national programs do not contemplate long-term follow-up or active search for those who do not return to controls, which means that recurrences are not treated in due time allowing lesions progression, making treatment even more difficult and reducing the possibility of cure for these patients.

This is also important from the point of view of the programs that must plan more effective actions and allocate additional resources for the treatment of relapses and complications due to the progression of the disease. Since there are not too many cases and the compromise can be severe and the consequences for the patient very serious, all cases of ML should be managed in a reference center by a multidisciplinary team that includes an ENT doctor and internists who collaborate in the control of the treatments that, if they are combined, can have more adverse effects, especially because the majority of ML are in older people who may have comorbidities.

S43. BRASILEISH. ANIMAL LEISHMANIOSIS: IS A CHANGE OF MIND NEEDED?

Leishmaniasis are non-contagious infectious diseases caused by different species of protozoa of the genus *Leishmania*, which present significant clinical and epidemiological diversities in the zoonotic and anthroponotic transmission cycles. The type of immune response presented by the animal after infection – cellular, humoral or mixed –, associated with other factors, such as genetics, age, sex, nutrition, co-infections, immunosuppressive conditions, concomitant ecto or endoparasites, parasite load and virulence of *Leishmania*, can contribute to greater susceptibility or resistance to the disease or even for the intensity of clinical manifestations. Several clinical manifestations can be verified, such as skin diseases, ophthalmopathies, nephropathies, hemorrhagic diathesis, among others. Thus, animals can be classified according to clinical staging, based mainly on quantitative serology, laboratory findings related to progressive kidney disease, the severity of the lesions and the analytical alterations presented.

S43-01: CANINE LEISHMANIOSIS BY *Leishmania infantum* IN PARAGUAY

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Canine Leishmaniosis (CL) is a parasitic, zoonotic disease caused by *Leishmania infantum* and transmitted by the bite of infected sandflies of the species *Lutzomyia longipalpis*. Since the first case of human Leishmaniosis detected in Paraguay in 1911, several cases have been reported in humans, feline, wildlife and canine, which is considered the main reservoir of the

parasite. Paraguay currently has a dog population of approximately 1.400.000 and according to the data obtained by the CEDIVEP laboratory, there is a serological prevalence of 41% in the last 19 years (2002-2021). The largest number of animals that were positive for ELISA or Immunochromatography tests was detected in Central department (92%) which is the largest urban population department of the country and has a better access to a veterinary service. Nowadays, CL is considered an endemic disease due to the changing climatic conditions, the presence of the vector *L. longipalpis* and the increased disorganized occupation in urban areas that leads the growth of the canine population. Several tools are currently available for diagnosing of CL, such as serological and molecular tests, which have been a great contribution to diagnosis and treatment monitoring. In recent years, diagnostic methods have improved with the use of conventional PCR and Isothermal PCR. Since 2016, CL vaccines are available having until today approximately 10.000 (<0.1%) vaccinated animals. It is used as an individual prevention method, combined with the use of repellents and insecticides. Currently non-human drug treatment is available to treat this disease under the supervision of a veterinarian. The high prevalence of CL observed in urban areas, shows the need to continue with strict epidemiological surveillance, health education for the community and responsible ownership education to pet owners by veterinary professionals and health authorities generating the correct application of preventive measures and control of this zoonosis in Paraguay.

Keyword CANINE LEISHMANIOSIS; PARAGUAY; *L. infantum*.

S43-02: CLINICAL STAGING AND MANAGEMENT OF *Leishmania infantum* INFECTION IN DOGS IN LATIN AMERICA

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Leishmaniasis are non-contagious infectious diseases caused by different species of protozoa of the genus *Leishmania*, which present significant clinical and epidemiological diversities in the zoonotic and anthroponotic transmission cycles. The type of immune response presented by the animal after infection (cellular, humoral or mixed), associated with other factors, such as genetics, age, sex, nutrition, co-infections, immunosuppressive conditions, concomitant ecto or endoparasites, parasite load and virulence of *Leishmania*, can contribute to greater susceptibility or resistance to the disease or even for the intensity of clinical manifestations. Several clinical manifestations can be verified, such as skin diseases, ophthalmopathies, nephropathies, hemorrhagic diathesis, among others. Dogs with leishmaniosis can be classified according to clinical staging, based mainly on quantitative serology, clinical signs and clinicopathological abnormalities related to progressive kidney disease.

S43-03: CANINE LEISHMANIOSIS CAUSED BY *Leishmania braziliensis*

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Canine leishmaniosis may be caused by a range of *Leishmania* spp., which are transmitted by various phlebotomine sand fly species. In the American continent, *Leishmania braziliensis* is the second most widespread causative agent of cutaneous leishmaniasis in humans and may also affect dogs and cats. Dogs infected by *L. braziliensis* typically present a cutaneous or mucocutaneous form of leishmaniasis. Skin or mucosal ulcers frequently localize on the ears, nose, and scrotal area. Skin lesions may be self-limiting, but may leave permanent scars, as in humans. In rural areas, skin lesions on the ears may be aggravated by stable flies (*Stomoxys calcitrans*), which are common parasites of dogs in many *L. braziliensis*-endemic areas. Considering that *L. braziliensis* and *L. infantum* may present overlapping distributions in some endemic foci, the use of molecular methods is highly recommended for a proper etiological diagnosis. There is limited information on the treatment of canine leishmaniosis by *L. braziliensis* and therefore further research on this matter is needed.

S43-04: CANINE AND HUMAN LEISHMANIASIS CAUSED BY *Leishmania infantum* IN LATIN AMERICA

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Leishmaniasis caused by *L. infantum* is a severe vectorial parasitic disease for both human and animals that affects many countries of South and Central America. Among animals, the dog is the specie most involved, and also plays an important role as the main urban reservoir of the parasite. In the early Twentieth Century, the presence of the parasite was described in humans, canines, felines and some wild animals in America. From the second half of this century the disease began to spread in many regions in sylvatic tropical areas first, then, in the last 60 years, it gradually became an urban problem. This was a consequence of the expansion of urbanization, deforestation, migration of both people and animals and adaptation of certain vectors to synanthropic life. Like other diseases, socioeconomic deterioration and poverty are the main factors that make adequate policies difficult to achieve, and facilitate the worsening of health conditions and treatment of both people and animals. Visceral Leishmaniasis is endemic in 13 countries of America being Brazil the country most affected. In 2020, 97% cases of human leishmaniasis (1933 people) were reported there, and the remaining 3% in other countries such Venezuela, Colombia, Paraguay, Argentina, Bolivia and Uruguay. Taking into account the environmental conditions of Central and South America, and the behavior of highly adaptable vectors, it is likely that, with the exception of Chile (due to its climatic and geographic characteristics), the rest of the countries that are not in this list of 13, either have undiagnosed presence of *L. infantum* or will have it in a short time. This is probably due to the expansion of the vectors´ distribution and the socioeconomic situation of the whole continent. Because of the COVID-19 pandemic, information in the last two years became less reliable. However, even if the update of statistics was affected to some degree, a progressive

and continue spreading of the disease has been observed across the continent, as we can confirm with newer foci of Leishmaniasis in areas like Northwest of Argentina, Eastern Bolivia near Brazilian border or the Colombian Pacific. Dogs are much more vulnerable to leishmaniosis, the number of animals affected anywhere is greater than the humans cases by several thousands, even though the geographical distribution is similar. Frequently, the emergence of canine cases precedes human ones and in a certain way, dogs act as sentinel specie to detect a focus of leishmaniasis in new areas. Unfortunately, reliable statistics of the real number of canine cases in different countries is not available, and information is very irregular However, in many regions is a matter of prioritary Public Health concern and a serious clinical problem for dogs.
Canine Leishmaniosis needs a One Health approach to manage and look for adequate strategies to control, involving:

- vector and environmental control.
- decrease of interaction between dogs and vectors by a correct use of repellents.
- an early and accurate diagnosis and treatment of sick animals and vaccination as a promising tool for dogs.

- education people about responsible ownership and environmental care.

Public strategies still may vary in different countries mainly in regard to the management of canine reservoirs. Still today in some of them, euthanasia is mandatory for positive animals.

In recent years, these policies are changing into account a more humane and rational approach, that includes adequate diagnosis and better vector control. In our countries specifically we have now advanced into the idea of treating sick animals with modern and scientific concepts, toward more effective and socially acceptable measures, regarding public health and animal welfare.

S43-05: DIAGNOSIS OF *Leishmania infantum* INFECTION IN DOGS IN LATIN AMERICA

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Leishmaniosis is a frequent infectious disease of dogs living in endemic areas, associated with important morbidity and mortality. In Latin America, canine leishmaniosis is mainly caused by *Leishmania infantum* and transmitted by the sand fly *Lutzomyia longipalpis*. The disease is widespread from Mexico to Argentina. The diagnosis of canine leishmaniosis is made by associating the patient's history, clinical signs and clinicopathological abnormalities. Most infected dogs are asymptomatic. However, the clinical presentation may be characterized by a wide spectrum of non-specific clinical signs that include dermatological, ocular, renal alterations, weight loss, lameness, epistaxis, lymphadenopathy, splenomegaly, among others. Clinicopathological abnormalities mainly comprise a mild to moderate normochromic normocytic anemia, thrombocytopenia, polyclonal gammopathy hyperglobulinemia, hypoalbuminemia and changing in kidney function markers. Co-infections by *L. infantum* and other canine vector-borne pathogens may confound clinical presentation, making diagnosis more challenging. Diagnostic tests are used to confirm the presence of *Leishmania* parasites, their DNA or anti-*Leishmania* antibodies. Positive serology may or may not indicate a current infection. Serological tests may also present false positives results, especially when low antibody titers are detected. The time of seroconversion is also variable leading to false negative results. Some countries in Latin America, including Brazil, still use the euthanasia of seropositive dogs as a way to control the disease. A positive parasitological or molecular test demonstrate that the dog is actually infected with *Leishmania* parasites. The tissue and disease stage can influence the results and the sensitivity of parasitological and molecular tests. The diagnosis of the disease (i.e., leishmaniosis) should rely on the presence of clinical signs, clinicopathological abnormalities and testing

results. In conjunction, all these data can be used for the clinical staging of canine leishmaniosis, which ultimately my guide therapeutic decisions, follow-up and prognosis.

S43-06: PROPOSALS FOR THE CONTROL AND MANAGEMENT OF CANINE LEISHMANIOSIS BY *Leishmania infantum* IN LATIN AMERICA

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One Health applied to the control of visceral leishmaniasis presupposes an expanded vision in the control measures of canine leishmaniasis (CanL), since the dog is considered as the main reservoir of *Leishmania infantum* for the infection of sand flies and consequent infection of other animals and humans. The public health service should recommend to all dogs, seropositive or not, the use of protective measures against sand fly vectors (mainly *Lutzomyia longipalpis*). In Brazil, insecticidal collars based on 4% deltamethrin or 4.5% flumethrin or 8.5% permethrin are licensed and, in some epidemiological situations, the Brazilian Ministry of Health distributes 4% deltamethrin collars to all dogs living in a given community every 6 months. In addition, public health officials should to advise dog owners to seek veterinary services for their dogs, including treatment of dogs with leishmaniosis or preventive vaccination (seronegative dogs only), where available. Collar application should be advised for both negative and positive dogs. Both public health veterinarians and clinical veterinarians should be aware regarding the recommendations for CanL prevention, sand fly control (including possible environmental measures to be adopted based on each local reality) and the management of infected dogs. In this way, the proposals for the control and management of CanL aim at human, animal and environmental health as an integrated and unique health.

S44 NEW HOPE FOR LEISHMANIASIS: HOW TO COMMUNICATE TO A BROADER NON-SCIENTIFIC AUDIENCE

1. A storytelling approach to social determinants of health related to leishmaniasis
Co-chair: Ximena Serrano Gil, Colombian Association of Journalism and Science Communication, World Federation of Science Journalism, Colombia

2. Strategic dissemination of research results: promoting positive impact on the population
Speaker: Efrain Rincón Alves, science journalist, Colombia

3.Health and inequalities: a story of coca deforestation and leishmaniasis
Speaker: Lina Pinto-García, Institute for Science, Innovation and Society (InSIS) - University of Oxford, Interdisciplinary Center for Development Studies (CIDER) - Universidad de los Andes, Colombia

4. Telling the story of leishmaniasis and war in Colombia: a book, a exhibit, and a policy brief

Summary

In the past two years became even more clear how important is that researchers can communicate their studies results to the wider public as this will illustrate the value that their work can bring to society. To ensure accuracy and to minimize the potential for misleading conclusions, many times not communicating seems to be the best option until final and big results are visible. But how can we keep the society up to date with science and research findings? What are the strategies or tools that researchers can use and what is the best way to communicate results?

While many scientists may be comfortable discussing their work with fellow professionals at conferences and seminars, communicating their work accurately to the wider public requires a different perspective, if only because journalists necessarily use different criteria for judging the interest and importance of new developments.

Researchers should be encouraged to talk about their work in an open and responsible way, balancing the need to maintain scientific rigor with the requirement that research should be communicated in a way that can be clearly understood by the wider public.

4. ORAL COMMUNICATION

4.1 CANINE LEISHMANIASIS

O1-01: MOLECULAR SURVEILLANCE OF LEISHMANIASIS SUGGESTS A LOW PREVALENCE BUT HIGH PATHOGENICITY OF *Leishmania infantum* IN DOGS FROM THE METROPOLITAN AREA OF BUCARAMANGA SANTANDER

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Dogs are the main reservoir of *Leishmania infantum* so their epidemiological surveillance is essential to control visceral leishmaniasis. Considering the paucity of studies of canine leishmaniasis in Colombia, the present study aims to determine the prevalence of *Leishmania* spp., in healthy and symptomatic dogs from the Metropolitan Area of Bucaramanga, Santander. The symptomatic group, corresponding to 26 dogs with differential diagnoses of canine leishmaniasis that attended clinical centers of this area. The healthy group corresponds to 215 dogs from the metropolitan area of Bucaramanga, Santander, that attend government schedules for rabies vaccination. In both groups, DNA was extracted from the blood samples, whereas a lymph node aspiration was additionally processed in the symptomatic group. Molecular diagnosis was performed using a PCR targeting the *Hsp70* gene of *Leishmania* spp., and the PCR products were

sequenced using Sanger methods. Molecular analyses showed a positivity rate of 27% (7/26), in the symptomatic group, which 11.5% (3/26), 11.5% (3/26), and 2.8% (1/26) came from Bucaramanga, Girón, and Floridablanca, respectively. All positive samples correspond to *L. infantum*. Higher positivity rate was detected in lymph node aspirations (6/26) compared with blood samples (4/26). Regarding epidemiological variables, four of the positive animals came from the shelters; signs of alopecia, cachexia, lymphadenitis, anemia, and lymphopenia, were associated with the infection. In two of the positive animals, death was reported weeks after diagnosis. Not positive animals were detected in the healthy group. The low positivity rate in the healthy group versus the high positivity of *L. infantum* in the symptomatic ones, suggests low infectivity of species accompanied by high pathogenicity, probably associated with genetic variants of the pathogen, as well as ecological conditions in the study area. These results should be taken into consideration when canine leishmaniasis control programs are implemented in Santander.

Keywords ZOONOSES; RESERVOIRS; VECTORS; TRYPANOSOMATIDS; LEISHMANIASIS

Financing Dirección Nacional de Investigaciones (DINAI), Universidad Cooperativa de Colombia

O1-02: *Leishmania infantum* AND CANINE MACROPHAGES: NEW INSIGHTS ON HOST-PARASITE IMMUNE CO-EVOLUTION

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Leishmania infantum is the aetiological agent of zoonotic visceral leishmaniasis (ZVL) that affects humans and dogs. One of the most interesting characteristics of this parasite is its ability to subvert the immune activation of macrophages (MØs), the host cells. These highly active phagocytic cells, which can destroy pathogens, are subverted to tolerate intracellular *Leishmania* parasites, allowing amastigote replication and favouring *Leishmania* dissemination in the mammal host. The liver has one of the large residents MØ populations of any other organ in the mammal body and is a target organ for *L. infantum* infection. However, liver infection is often described as self-contained and able to restrain parasite replication. Thus, the aiming of the present study was to compare, the innate immune response of two different macrophage lineages: the blood macrophages and Kupffer cells (KC), the resident liver macrophage population, regarding *L. infantum* infection. Therefore, blood-MØs and KCs were isolated from healthy dogs and exposed *in vitro* to virulent *L. infantum* promastigotes and amastigotes, respectively. Gene expression of innate immune receptors

(NODs and TLRs), as well as generation of pro- (IL-12 and TNF-α) and anti-inflammatory (IL-4, IL-10, and TGF-β) cytokines, were analysed by Real-Time PCR. Cellular viability, nitric oxide production, and scan electron microscopy (SEM) were also performed. Our results showed that blood-MØs exposed to *L. infantum* rapidly phagocytosis the parasites and activate innate immune receptors, generating high amounts of cytokines. However, after 5 h of infection, blood-MØs exhibited an overall reduction of innate immune receptors and cytokine gene expression, clearly showing the subversion of cellular immune mechanisms. Remarkably, blood-MØs showed an extended lifetime enabled by parasite intracellular infection. On the other hand, KCs presented a steady expression of innate immune receptors (NOD1, TLR2, and TLR4) and cytokine generation (IL-12, IL-10, and TGF-β). Moreover, SEM analysis showed evidence of macrophage extracellular traps (METs) emitted by activated KCs. Altogether, our findings suggest that *L. infantum* can take advantage of the natural predisposition of blood-MØs to perform phagocytosis to rapidly subvert the activation cell's immune mechanisms and guarantee its survival inside the host cell. On the other hand, KCs appear to be more efficient in managing parasite infection, probably contributing to the ability of the liver to naturally restrain the dissemination of *Leishmania* parasites.

Keywords *Leishmania infantum*; INNATE IMMUNITY; KUPFFER CELLS; BLOOD-MACROPHAGES; HOST-PARASITE INTERACTION

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O1-03: INCREASE IN THE OCCURRENCE OF CANINE VISCERAL LEISHMANIASIS AND IDENTIFICATION OF EPIDEMIOLOGICAL FACTORS IN CAMAÇARI - BA FROM 2011 TO 2015

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Urbanization is a process that affects several relevant factors such as biodiversity, basic sanitation, and socioeconomic aspects favoring Visceral Leishmaniasis (VL) occurrence. Following the progression of urbanization, the VL vector's habitat has been gradually adapted to a new environment. In this process, dogs became *Leishmania infantum* main reservoir in urban areas, therefore being important for Public Health concerns. The higher prevalence of Canine Visceral Leishmaniasis (CVL) leads to an increase in human disease. The association between urbanization and appearance as well as development of diseases such as VL and CVL is closely related to the unplanned and disordered urbanization process. We aimed to evaluate possible associations between CVL distribution, environmental and socioeconomic changes concerning urbanization in Camaçari – BA, Brazil in a period of two years. The spatial and epidemiological data used were gathered from two previous epidemiological studies, conducted in different periods: period 1 from 2011 to 2012, analyzing 91 dogs (37 positive and 54 negative cases for CVL), and period 2 from 2014 to 2015, analyzing 463 dogs (288 positives and 175 negatives). Spatial analysis of CVL cases, *Lutzomyia*

longipalpis capture sites, and urbanization data were performed. Additionally, epidemiological data were analyzed in both periods using data from surveys conducted with animal owners. Domestic and sociodemographic characteristics of households were evaluated. Results showed a augmentation of 21.5% ($p<0.05$) in CVL frequency between the periods analyzed (40.7% in period 1 and 62.2% in period 2). Over periods 1 and 2, it was possible to identify, among domestic characteristics of the households, a lower frequency of chickens' coops (from 56.3% to 34.5%) ($p<0.05$). In the sociodemographic characteristics, it was possible to identify an increase in the number of households that receive government incentives (from 20.7% to 38.8%) ($p<0.05$). Although the data are still preliminary, our results show that there has been an increase in CVL cases over the two periods and that there was a possible decrease in socioeconomic conditions of the residents from Camaçari, which may be related to the CVL spreading. Our work will help to identify areas at higher risk of VL and CVL dissemination, providing a better comprehension of the relationship between the urbanization process and the disease in order to perform more specific and efficient interventions.

Keywords CANINE VISCERAL LEISHMANIASIS; URBANIZATION; CASES DISTRIBUTION; SPATIAL ANALYSIS

Financing Fapesb, CAPES and IGM-FIOCRUZ

O1-04: ANTIBODY RESPONSE TO *Leishmania* AND TO *Phlebotomus perniciosus* RECOMBINANT SALIVARY ANTIGENS IN DOGS OVER ONE YEAR IN A LEISHMANIASIS ENDEMIC FOCUS

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Canine leishmaniasis caused by *Leishmania infantum* is endemic in several countries of Latin America, and the Mediterranean Basin, including regions of southwestern Europe, where the main vector is *Phlebotomus perniciosus*. During the blood meal, immunogenic components present in the sand fly saliva are inoculated into the vertebrate host, causing the development of anti-saliva antibodies, whose detection has proven to be a useful epidemiological biomarker to monitor the exposure of hosts to vectors and can be used to estimate the risk of *Leishmania* infection. The aim of this study was to evaluate the specific antibody response to *Leishmania* and to *P. perniciosus* salivary antigens in dogs over one year in a leishmaniasis endemic focus *where P. perniciosus* density is considered to be bimodal with a small peak in May and a major in September. Forty-two shepherd and hunting dogs from an endemic area of leishmaniasis (Algarve region, Portugal) were screened before, during (April to October) and after one sand fly season. Detection of anti-*Leishmania* antibodies was done using IFAT while the exposure to *P. perniciosus* salivary recombinant antigen rSP03B was measured by ELISA. *P. perniciosus* salivary antibodies were detected in the sera of 36 (86%) of the screened dogs at least one time during the follow up. The overall median values of ELISA absorbance (ABS) was 0.8. ABS decreased from the end of the season (December 2018; ABS = 0.9) until the beginning of the next sand fly activity (May 2019; ABS = 0.7),

increased in June and decreased thereafter until August, with a new, smaller increase at the end of season (October 2019; ABS = 0.8), followed by the lowest ABS obtained before the beginning of the following season (March 2020; ABS = 0.6). Antibodies to *Leishmania* were detected in a single dog at the beginning of the study and in two dogs throughout the sand fly season, indicating an incidence risk of 5%. No association between the presence of antibody response to recombinant salivary antigen and to *Leishmania* was found. Seasonal exposure of dogs to sand flies led to antibody response fluctuations related to the period of activity of *P. perniciosus* with a decline after the end of the biting season. Our results reinforced the usefulness of anti-saliva antibodies as biomarkers for evaluating the exposure to phlebotomine sand flies but did not support their potential use as biomarkers of *Leishmania* exposure/infection

Keywords DOG; EXPOSURE; *LEISHMANIA; Phlebotomus perniciosus;* SALIVA

Financing Fundação para a Ciência e a Tecnologia, I.P. (GHTM-UID/Multi/04413/2013; IF/01302/2015)

O1-05: MIR-148A AND MIR-21 REGULATE IMMUNE RESPONSE IN CANINE LEISHMANIASIS

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Leishmaniasis is a often neglected and potentially lethal disease when left untreated. Canine leishmaniasis (CanL) is a serious public health problem because infected dogs are capable of transmission of the *Leishmania* protozoan to humans through the phlebotomine vector. The progression of CanL, is related to effective immune response suppression that can be associated with small fragments of RNA called microRNA (miR), which can alter the proteins translation and regulate various biological functions of cells. We evaluated miRNA expression in splenic leukocytes (SL) from dogs naturally infected with *Leishmania infantum* and developing leishmaniasis (CanL; n = 8) compared to healthy dogs (n = 4). This study was approved by the Committee for Ethics in Animal Experimental Research (COBEA), with the approval of the Committee for Ethics in Animal Use (CEUA) of São Paulo State University (UNESP), School of Veterinary Medicine, Araçatuba,S.P, Brazil. Microarray analysis showed increased expression of miR 21, miR 148a, miR 7 and miR 615, and downregulation of miR 150, miR 125a and miR 125b. Real-time PCR validated the differential expression of miR 21, miR 148a and miR 615. In silico analysis showed that miR21 and miR-148a increased in splenic leukocytes of dogs with leishmaniasis and can affect several pathways involved in the regulation of the immune response. Mimics and inhibitors of miR-148a and miR-21 were used *in vitro* to transfect splenic leukocytes from dogs with CanL. After transfection, expression levels of the target proteins related to immune system were measured. The miR-148a mimic decreased the expression of iNOS in the

splenic leukocytes and it also decreased TNF-α, IL-12, IL-6 in culture supernatant of splenic leukocyte from dogs with CanL. Decrease of miR 148a in splenic leukocytes, by means of transfection with a miR 148 Inhibition decreased the parasitic load in splenic leukocytes from dogs with CanL. The miR-21 mimic decreased CD69 expression in splenic leukocytes from CanL. Further, decrease of miR 21 in splenic leukocytes, by means of transfection with a miR 21 inhibitor, decreased IL-10, increased the IL-12 cytokine and the T-bet/GATA-3 ratio, and decreased parasite load on splenic leukocytes of dogs with CanL. These findings suggest that miR-148a and miR-21 regulates inflammation and microbicidal activity the immune response in CanL.

Keywords *Leishmania chagasi*; MICRORNA; DOGS; SPLEEN; CYTOKINES; NITRIC OXIDE

Financing FAPESP (2018/17261-5)

O1-06: STUDY OF THE OCCURRENCE AND SPATIAL DISTRIBUTION OF CANINE LEISHMANIASIS IN ITABERABA, BRAZIL FROM 2012 TO 2018

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In Brazil, dogs are of great importance in the epidemiology of visceral leishmaniasis (VL) as they are considered the main domestic reservoir for *Leishmania infantum* parasites. Studies have already shown that the appearance of human cases is preceded by the emergence of the disease in dogs. Within the context of one health, monitoring the occurrence of canine leishmaniasis (CanL) is essential for controlling this zoonosis in both the human and canine populations. The main objective of this project was to describe the occurrence and spatial distribution of CanL in Itaberaba from 2012 to 2018 and to correlate the findings with the social and demographic situation of the municipality. Itaberaba is located in the state of Bahia, in the Northeast region of Brazil. Data from cases of CanL, confirmed by serological protocol, detected by the Health Department of the municipality were used. The characteristics of human VL cases that occurred in the same period within the geographic limits of the municipality were also evaluated. The addresses of the human and canine cases included were georeferenced. A descriptive analysis of the main demographic characteristics found in the affected dogs was carried out, and the occurrence of CanL was evaluated by year and by region/district of the municipality. Spatial analyzes and maps were produced using the free software QGIS. The spatial analysis was carried out on a cartographic basis of the municipal network and census sectors obtained in the Brazilian Demographic Census of the year 2010. Additionally, satellite images from different years of the territory of

Itaberaba were acquired to evaluate environmental characteristics, such as the vegetation cover index. According to data from the Health Department of Itaberaba, from 2013 to 2018, there were seven cases of VL, with the death of one child in 2013. The municipality lacks a zoonosis control center, so the investigations to detect CanL cases are performed only on spontaneous demand from the population or in the presence of dogs with a clinically suggestive CanL. At this point, we evaluated 2038 reports from 2012 and 2015. CanL occurrence was 21% (95/446) in 2012, 32% (274/846) in 2013,44% (254/574) in 2014, and 5,8% (10/172) in 2015. We are still analyzing data from 2016 to 2018. CanL is considered endemic in Itaberaba, with high occurrence rates through the years. In the spatial distribution, it was possible to observe the poorest locations as the most affected. This is the first official report regarding the occurrence of CanL in Itaberaba. The systematic evaluation of reported cases in the municipality may provide important information about the areas of greatest risk, determining which environmental characteristics may be influencing the occurrence of this zoonosis. The results of this project may directly influence the implementation of public policies to control CanL in the region, which may indirectly reduce the incidence of human disease.

Keywords CANINE VISCERAL LEISHMANIASIS; ZOONOSIS; CASES DISTRIBUTION; SPATIAL ANALYSIS

Financing Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES 001)

O25-01: LABORATORY VALIDATION OF AN ELISA METHOD TO MEASURE CIRCULATING IMMUNE COMPLEXES LEVELS TO MONITOR THE PROGRESSION OF CANINE VISCERAL LEISHMANIASIS

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Canine visceral leishmaniasis (CanL) is a global zoonosis caused by *Leishmania infantum*. Susceptible dogs develop an ineffective immune response that leads to the formation of circulating immune complexes (CIC), which are aggregates of *Leishmania* proteins and anti-*Leishmania* immunoglobulins. Their deposition in several tissues causes some of the most severe clinical manifestations of CanL. There is a need of biomarkers able to confirm *Leishmania* infection and to monitor disease progression during treatment in the veterinary practice. We recently described a *Leishmania*-specific method to isolate and quantify CIC in dog serum samples, demonstrating a positive correlation between CIC levels and degree of pathology. The objective of this study was to validate this new method as a tool to predict and monitor disease progression and treatment efficacy in CanL. Serum samples were collected from dogs, naturally infected with *L. infantum*, and grouped according LeishVet classification as: healthy non-infected (n=36); healthy infected (n=8); sick stage I (n=7); sick stage II (n=8); and sick stage III/IV (n=7). CIC were isolated from sera by polyethylene glycol (PEG) precipitation. An enzyme-linked immunosorbent assay (ELISA) was used to measure CIC levels. Plates were coated with Soluble *Leishmania* Antigen (SLA) and then incubated with PEG-precipitated CIC. The validation of the method was performed according to international quality guidelines including the parameters specificity, precision, dilution linearity and robustness. Data obtained were analyzed using GraphPad-Prism software. The cut-off was established (0.274) and only results above

the cut-off were considered as positive. Concerning specificity, all analyzed sera showed a mean OD value below the cut-off, confirming that 100% of samples were negative. Results of the precision studies revealed CVs below the described limits in all cases: intra-assay (CV <10%), inter-assay (CV <10% for high reactivity samples, CV <25% for medium reactivity samples and CV <35% for low reactivity samples) and intermediate precision (CV <20% for high reactivity samples, CV <30% for medium reactivity samples and CV <40% for low reactivity samples). Linear relationship between OD results and CIC levels was analyzed. All samples fit the 4-parameter sigmoidal model and the R^2 value was ≥0.99 in all cases. Regarding robustness, there were no significant differences ($p>0.05$) neither when samples were tested using three different batches of SLA nor when different incubation times were used, proving that these variations do not significantly disturb the reactivity of the experimental samples. CIC have been proposed as biomarkers with potential diagnostic and prognostic value for CanL, and we have developed a method for isolation and quantification of CIC in serum samples. This ELISA method was validated with acceptable results concerning linearity, specificity, precision and robustness that make it a practical clinical tool to support veterinarians in the management of CanL, not only to confirm infection but also to monitor the treatment. CIC measurement could be including in the panel of analyses for *L. infantum*-infected dogs, allowing clinicians to establish proper disease management in CanL.

Keywords *Leishmania infantum*; CIRCULATING IMMUNE COMPLEXES; BIOMARKERS; CANINE LEISHMANIASIS

O25-02: IMMUNE RESPONSE TO *Leishmania infantum* IN DOGS: A WAY TO UNDERSTAND ASYMPTOMATIC CARRIAGE IN HUMAN?

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Leishmania infantum is found in Latin America and in the Mediterranean Basin where dogs are the main reservoir. Mechanisms of the immune system in dogs are poorly understood during visceral leishmaniasis and we do not know the differences in host-pathogen interactions that favour asymptomatic carriage in humans, contrary to the development of visceral leishmaniasis in dogs. *L. infantum* recombinant parasites expressing the Green Fluorescent Protein (GFP) were used to infect the canine macrophage line DH82 (ATCC number CRL-10389). The infection was confirmed by Fluorescence-activated Cell Sorting (FACS) and confocal microscopy. We performed a canine-specific cytokine array kit to detect patterns of cytokine expression in macrophages during *L. infantum* infection. The relative expression of 40 cytokines was measured by chemiluminescence on DH82 cell lysates after infection with *L. infantum* or not. Among the cytokines whose level of expression was increasing, the following showed at least a two-fold increase after infection: Cystatin C, HGFR, interferon γ, IL-1α, IL-21, IL-6, MCP-1, CXCL1, TNF R1, IGFBP-2 and Trappin-2. Most of them are involved in the inflammatory response. Interferon γ is well known to participate in the defence response to microorganisms and macrophage differentiation and activation, IL-1α is produced by activated macrophage and is identified as an endogenous pyrogen, IL-21 can stimulate interferon γ production, IL-6 is produced by macrophages and dendritic cells and is involved in the recognition of pathogens through Toll-Like Receptors, MCP-1 and CXCL1 exhibit a chemotactic activity for monocytes and neutrophils, respectively. Conversely, the following cytokines showed at least a two-fold decrease after infection: Galectin-3, GASP-1, IL-13, IL-17A and RAGE. The cytokine GASP-1 is known to target receptors for degradation in lysosomes, IL-17A has an activity of positive regulation in inflammatory cytokine

production and RAGE is involved in the negative regulation of IL-10. In total, those results show the complexity of the balance of cytokines during *L. infantum* infection in canine macrophages. The down regulation of GASP-1 is very interesting because of its role in lysosomes and it should be further evaluated, as the parasitophorous vacuole become a phago-lysosome during the infection. The absence of increase of IL-1β after infection is also to notice. Indeed, inflammasomes and particularly NLRP3 has not been proved to be involved in *L. infantum* infection contrary to other *Leishmania* species. IL-1β is largely produced by NLRP3, so this result tends to confirm the absence of implication of NLRP3 in *L. infantum* infection, as it has been evocated in some publications.

Keywords *Leishmania infantum*; DOG; IMMUNE RESPONSE; CYTOKINE

O25-03: IMMUNOLOGICAL PROFILE AND PARASITE LOAD IN NATURALLY INFECTED DOGS BY *Leishmania* SPP. IN THE MUNICIPALITY OF OVEJAS, COLOMBIA

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Leishmaniasis are a group of diseases caused by protozoan parasites of the genus *Leishmania,* which in America are transmitted by sandfly of the *Lutzomyia* genus. In Colombia, leishmaniasis are considered a Public Health issue and it's estimated about 10 million people are at risk of acquiring the infection. The Montes de María, on the Caribbean coast is one of the main foci of the country, with reports of cases of cutaneous and visceral leishmaniasis. Dog, *Canis familiaris,* is considered as the main domestic reservoir of *Leishmania infantum,* causal agent of visceral leishmaniasis in Europe and America, and it is possible that it also plays a role in the transmission cycle of other species of the *Leishmania* genus. On the Colombian Caribbean, *C. familiaris* has been found infected with *L. infantum,* besides with *L. braziliensis* and *L. guyanesis,* the latter are the causal agent of cutaneous leishmaniasis in humans. However, there's no experimental evidence of the infective capacity for the vector insect. The objective was to verify if the infected dogs present in the municipality of Ovejas are infective to the vectors, as well as to determine the parasite load and to identify other aspects such as immune response from these dogs, which would evidence the potential infective character for the vector; this could allow to elucidate the role that *C. familiaris* has in the transmission cycle of species of the genus *Leishmania*. On first instance, a diagnosis of visceral canine leishmaniasis

was made with serological and molecular test, obtaining an infection rate of 44,14% (IC 95% 0,3525- 0,5343); an association between seropositive canines and an age over 15 months was found. Likewise, the parasite load was evaluated from the peripheral blood by a quantitative PCR and obtained for 15 canines, with values from 1,80 to 22866,88 parasites/ml. Then, 12 xenodiagnosis with female of *Lu. evansi,* known vector of *L. infantum* on the Montes de María region, were made, which allowed to establish that, in fact these canines are capable to transmit the parasite to the sandfly vector, with infection frequencies from 5,26 to 31,25 on dogs with and without clinical signs of leishmaniasis. At last, assisted with luminex technology, 12 proteins were evaluated (interleucines, chemokines and growth factors) on these canines, and it was established that seropositive canines present higher concentrations of TNF-α; positive canines by PCR presented greater concentrations of IL-15, IL-8 and GM-CSF. It was found besides those dogs infected with *L. infantum* presented higher concentrations of IP-10 and GM-CSF. It is concluded that *C. familiaris* acts as reservoir of *L. infantum,* -without ruling out its place as potential reservoir of other species of parasites of *Leishmania* genus- on the urban area of the municipality of Ovejas. It is expected that the generated knowledge helps Public Health entities to establish an effective control of domestic reservoirs which participate in the cycle of leishmaniasis in this mix focus of the disease

Keywords CANINE LEISHMANIASIS; PARASITE LOAD; INFECTIVITY; CYTOKINES; CHEMOKINES

O25-04: MOLECULAR IDENTIFICATION OF *LEISHMANIA* SP. FIELD ISOLATES FROM DOGS WITH VISCERAL LEISHMANIASIS REVEALS COINFECTION WITH PARASITES FROM *Viannia* AND *Leishmania* SUBGENUS

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Leishmaniasis is an important health problem in tropical and subtropical countries including Brazil. The manifestation of the disease depends on the infecting species and host immune response. *Leishmania (L.) infantum* is associated with the visceral form of leishmaniasis in dogs and humans, and some recent studies also point to a possible role of *L. (L.) amazonensis* in this clinical manifestation. Coinfections involving different *Leishmania* species are, however, not so frequently described in the literature. Thus, surveillance of seropositive dogs in endemic areas with clinical manifestation of visceral leishmaniasis followed by the isolation and characterization of *Leishmania* spp. isolates represent an important approach for a better understanding of the biological behavior of these parasites. In this study, dogs from São Joaquim de Bicas and Belo Horizonte cities, endemic regions in Minas Gerais state, are being constantly evaluated clinically and their blood samples submitted to serological assays such as rapid test (SNAP *Leishmania* – IDEXX, Maine, USA) and an in-house ELISA, which employs rKDDR as primary antigen to detect *Leishmania* infection. Twenty positive dogs in at least one serological test were submitted to a bone marrow aspiration for parasite isolation. gDNA samples were then submitted to subspecies-specific PCR targeting a conserved region of *Leishmania* kDNA minicircle to differentiate *Leishmania* and *Viannia* subgenus. In four samples, a pattern of coinfection with *Viannia* and *Leishmania* subgenus was observed, and in one of them *Viannia* subgenus

profile was detected, a result that was confirmed by sequencing the PCR products. For the remained samples, the amplicons obtained indicated the occurrence of *L. infantum* infections. These data demonstrate the occurrence of atypical infections involving different *Leishmania* species in naturally infected dogs and are of great relevance for a better understanding of epidemiological aspects and infection dynamics of *Leishmania*, which may contribute to the design of better control strategies in endemic regions.

Keywords VISCERAL LEISHMANIASIS; DOGS; *Leishmania* sp. COINFECTION

O25-05: CYTOKINE GENE EXPRESSION AND LYMPHOCYTE IMMUNOPHENOTYPING IN DOGS WITH LEISHMANIOSIS

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Canine leishmaniosis (CanL) caused by *Leishmania infantum* is a zoonotic disease of worldwide concern, endemic in more than 70 countries. The impact of *Leishmania* spp. infection in the host strongly depends on its immune competency, and recent studies have suggested that immune response is more complex than the dichotomic idea of protective Th1 *versus* Th2 response. Thus, it was proposed that *Leishmania* spp. develop mechanisms to modulate the host immune response, enabling its survival and dissemination and that immune response may differ among different compartments. Therefore, this study aimed to evaluate the immune response of dogs with leishmaniosis by analysing the profile of cytokines and subsets of $CD4^+$ and $CD8^+$ T cells in peripheral blood (PB), lymph node (LN) and bone marrow (BM). Two groups of dogs were established: one comprising twelve dogs diagnosed with CanL in LeishVet stages I/II or Stage C of the Canine Leishmaniasis Working Group classification system; and a control group of ten clinically healthy dogs. Following diagnosis, clinical signs, haematological, biochemical parameters, urinalysis results, and anti-*Leishmania* antibody titres using IFAT, mononuclear cells from PB, popliteal LN and BM were collected to evaluate the gene expression of IL-2, IL-4, IL-5, IL-10, IL-12, TNF-α, TGF-β and IFN-γ by qPCR. Furthermore, immunophenotyping of these cells was performed by flow cytometry, using monoclonal antibodies anti-CD45, CD3, CD4, CD8, CD25 and anti-nuclear

factor FoxP3. Dogs with CanL showed an overall increase of IFN-γ, and a decrease of TGF-β gene expression. However, IL-2, IL-12, TNF-α, IL-4, IL-5 and IL-10 showed variations among the different tissues analysed. Diseased dogs also showed an increase in the frequency of $CD8^+$ and $CD4^+CD8^+$ double-positive T cells in all tissues. In PB there was a decrease in $CD4^+$ and increase in $CD4^+CD25^+FoxP3^+$ and $CD8^+CD25^+FoxP3^+$ T cells, the latter also increased in BM. $CD4^+CD25^-FoxP3^-$ T cells showed an evident decrease in PB and BM. Dogs with CanL also had lower CD4/CD8 ratios in PB (<1) and LN (≈1) when compared with healthy dogs (≈2). These findings reinforce previous studies indicating that *L. infantum* may be capable of manipulating the dog's immune system, preventing the development of an efficient protective response, and enabling the parasite's survival. The presence of $CD8^+$ Treg cells in CanL was confirmed in this study, possibly representing another mechanism of immune regulation to maintain *Leishmania* infection which should be further investigated. The increasing proportion of $CD4^+CD8^+$ double-positive cells found in CanL dogs is gsimilar to that found in studies involving other chronic diseases and may possibly be an interesting tool for monitoring treatment predicting potential relapses. The current study highlights that although Th1 response is important to resistance, diseased dogs may present a mixed Th1/Th2 or Th1/Treg profile. It also supports the increasing evidence of compartmentalized immunity, despite some similarities amongst different tissues such as increased IFN-γ and $CD8^+$ T cells, reflecting a pro-inflammatory and cytotoxic response in CanL patients.

Keywords CANINE LEISHMANIOSIS; CYTOKINES; IMMUNOPHENOTYPING; LYMPHOCYTES

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4.2 DIAGNOSIS - TREATMENT AND RESISTANCE - CLINIC

O7-01: RELATIONSHIP BETWEEN THE PRESENCE OF PARASITIC DNA IN THE NASAL MUCOSA AND ASPECTS OF THE SKIN LESION IN AMERICAN TEGUMENTARY LEISHMANIASIS

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American Tegumentary Leishmaniasis (ATL) is an infectious disease caused by different species of *Leishmania* protozoa. Cutaneous leishmaniasis (CL) usually presents ulcers on the skin, which may spread to the mucosa in about 4% to 15% of cases within 3 years of disease. Considering the risk factors are not clear, the objective of this study is to identify and describe the relationship between the morphological aspects of CL skin lesions and the presence of Leishmania DNA in the nasal mucosa. The study design was analytical, observational, and cross-sectional. The sample consisted of 75 patients diagnosed with ATL and cutaneous manifestations. Pretreatment photographs of the lesions were taken and nasal swabs were collected. Data were collected such as age, sex, disease evolution, presence or absence of mucous symptoms, number of skin lesions, the sum of lesion areas, area of erythema, infiltration area, ulcer area and number of body segments affected by the lesions. The total lesion areas, ulcer areas, infiltration and

erythema were calculated using the Image J software (Madison, WI, USA) - ORC Forensics Scale PT-041 (Oregon City, OR, USA). Statistical methods included Student's t-test, Wilcoxons rank sum, and Fisher's exact test. There was a median of 3 months of disease evolution, 1 lesion, and 1 affected body segment. 24% of the patients had positive PCR on the nasal swabs and 40% had lesions in the upper limbs. 66.11% of patients with positive nasal swabs had lesions in the upper limbs and 33.33% negative nasal swabs had lesions in the upper limbs. The prevalence ratio of positive nasal swabs between patients with lesions in the upper limbs and those without lesions was 1.18 (95% CI, 1.01 – 1.38), p-value < 0.05. We observed a higher rate of early dissemination of Leishmania to the nasal mucosa than that described in the literature, the areas of infiltration, macula and erythema were higher in patients with dissemination of Leishmania to the nasal mucosa, but the association was not significant, perhaps because of the restricted number of patients. In this study, a higher prevalence of positive nasal DNA was attributed to patients with upper limb lesions. The results reinforce the need to increase the sample to study these likely clinical risk factors that may help in the challenging diagnosis and treatment of the mucosal form.

Keywords CUTANEOUS LEISHMANIASIS; MUCOSAL LEISHMANIASIS; PROGNOSIS; DNA; NASAL SWAB; RISK FACTORS

Financing CNPq process 307358/2017-8 and 404594/2021-2

O7-02: FIRST REPORT OF *Leishmania donovani* ISOLATED ON CHILDREN WITH CLINICAL PRESENTATION OF ENDEMIC KAPOSI SARCOMA IN MOKOLO, CAMEROON

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In Cameroon, the 2010 WHO Expert Committee considered only cutaneous leishmaniasis due to *L. major* endemic. *L. donovani* in humans has only been found endemic in East Africa and Asia. Some 20 years ago, a sero-epidemiological survey found anti-Leishmania antibodies but no cases of visceral leishmaniasis in Cameroon. In February 2017, an epidemic of pediatric eruptive fever of unknown origin was detected in the North and Far North Regions of Cameroon and was notified to WHO. The country reported 52 suspected cases including 20 deaths (40% fatality rate) and 24 lost to follow/up. Most of the cases (40%) are from Mokolo. Most of these mysterious died children had persistent fever, hepato-splenomegaly, lymphadenopathies, anemia and a skin eruption with mainly acral distribution. Investigations on treponematosis, rubella, measles, enterovirus, HIV, CMV, EBV and Pox virus were negative. The Institute Pasteur of Cameroon diagnosed cutaneous leishmaniasis on skin biopsies in seven patients. However, sera analysis (serology and sera PCR) by WHO collaborating center (Institute of Health Carlos III of Madrid) turned out negative to leishmaniasis, histoplasmosis, aspergillosis and other emerging and rare mycosis. In July 2017, a field mission was conducted in Mokolo District Hospital. Active case detection was carried out in the villages before the mission. The case definition was: child under 5 with skin eruption

essentially palmoplantar and cephalic, persistent fever for more than two weeks, that does not respond to treatment. Of the eight patients recruited initially three were included for clinical and laboratory examination. The Leishmania species identification was done in August 2017 through conventional and Real Time PCR to detect the presence of Leishmania DNA in samples obtained from glass slides previously stained with Giemsa. One part of the tissue material fixed on the glass slide was detached using saponin according to the protocol previously standardized in the PECET Lab. This material was deposited in 1.5 vials and the DNA was extracted. For the DNA amplification two PCR protocols were carried out. One PCR reaction was performed using a conventional PCR following the protocol described by Montalvo et al, (2017) and the other, by real-time using a fragment of the DNApol2 gene as PCR target and the Type-it HRM PCR Kit (Qiagen). The three patients had lymphedema of the legs, violaceus papulo-nodules and/or plaques of the extremities and lymphadenopathies. The clinical diagnosis was Endemic African Kaposi Sarcoma. The smear slides of these violaceus plaques showed Leishmania amastigotes and PCR analysis were consistent with *L. donovani*. The findings of *L. donovani* confirmed the results of previous biopsies reported by the Pasteur Institute of Cameroon where Leishmania amastigotes were seen. Probably, most of the patients who died had Kaposi sarcoma parasitized with Leishmania of the violaceus nodules and plaques. These findings warrant further and detailed investigations in the Far North of Cameroon to properly characterize the presence and implications of *L. donovani* in humans.

O7-03: OBESITY INCREASES THE TISSUE INFLAMMATORY RESPONSE AND INFLUENCES THE SEVERITY OF CUTANEOUS LEISHMANIASIS

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Obese cutaneous leishmaniasis (CL) patients have a high rate of therapeutic failure to meglumine antimoniate (Sb^5). Here we characterize the immune response in both blood and tissue and the histopathologic features of the skin and of the adipose tissue in obese and lean CL patients. Moreover, we evaluate if extend the treatment with Sb^5 for 30 days would improve the cure rate of these patients. This is a mixed study with a cross-sectional component comparing immunologic and histopathologic features of obese and lean CL patients and a trial comparing response to therapy of obese CL treated with Sb^5 for 20 or 30 days. Obese patients had a body mass index (BMI) greater than 30 and lean patients had BMI between 20 and 25. Participants were 30 obese and 30 lean CL patients with diagnosis confirmed by identification of DNA of *L. braziliensis*. Skin and adipose tissue biopsies were performed with 4mm punch after anesthesia and incubated for 48hours in culture medium. Peripheral blood mononuclear cells (PBMC) were obtained by gradient centrifugation, adjusted to a concentration of $3x10^6$ cells/ml in RPMI 640, stimulated with 5µg of soluble leishmania antigen and incubated for 48 hours. The supernatants were collected and cytokines and adipokines levels were measured by ELISA. Lean CL patients were treated with Gucantime (Sanofi-Aventis) in the dose of 20mg/Kg/day

for 20 days. Obese patients were randomized and treated with Glucantime in the dose of 20 mg/Kg of weight for 20 or 30 days. Cure was defined as complete reepithelization of the lesion on day 90 after initiation of therapy and failure by the presence of an active ulcer or permanence of raised borders in a healed lesion. There was no difference between obese and lean patients regarding the age, duration of illness and number of lesions. The inflammatory reaction was more intense in obese than in lean patients. Macrophages were more frequent in the adipose tissue of obese and leishmania infected macrophages were found in the adipose tissue of obese CL patients. The production of cytokines (IFN-γ, IL-1β, IL-5, IL-6, TNF, IL-17 and IL-10) were similar in supernatants of PBMC obese and lean patients ($P>0.05$). Leptin levels were higher in obese than in CL patients. The failure rate to meglumine antimoniate was higher in obese than in lean CL and extend the therapy for 30 rather than 20 days did not enhance response to therapy. The immune response in PBMC and in the tissue of obese and CL patients were similar with a predominant type 1 immune response. The leptin levels were increased in lean and obese patients CL patients although higher in the late group. The adipose tissue contributes for the inflammatory response of CL and the presence of macrophages and amastigotes in the adipose tissue release mediators that promote a more exuberant inflammatory infiltrate making CL lesions more severe in obese and associated with high rate of failure to therapy.

Keywords CUTANEOUS LEISHMANIASIS; TEGUMENTARY LEISHMANIASIS; OBESITY; OBESE CUTANEOUS LEISHMANIASIS

Financing Brazilian Research Council (CNPQ), Fundação De Amparo À Pesquisa Do Estado Da Bahia (FAPESB), National Institutes Of Health (NIH)

O7-05: ATYPICAL LEISHMANIASIS CAUSED BY *Leishmania infantum* IN A HIV PATIENT WITH SECONDARY VISCERALIZATION (KALA-AZAR): FIRST CASE REPORT IN PANAMA

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Leishmania infantum has a specific geographical distribution. In Latin America it is endemic in 12 countries; The highest number of cases are Brazil, Honduras, Colombia, and Venezuela. The new world *L. infantum* causes subclinical manifestations and active visceral leishmaniasis. However, in Central America, it causes atypical cutaneous leishmaniasis (ACL). We present the first case report of Meso American Kala-azar in a patient with AIDS in Panama. A 35-year-old male with a previous diagnosis of HIV infection without antiretroviral treatment seeks medical attention for a 6 month history of wasting, daily fever, chills, and abdominal pain in "L" shape. It also refers a non-painful nodular skin lesion on the right forearm of 1 year of evolution. The patient is from Managua, Nicaragua and has lived in Panama for the last 15 years, where he is a smuggler that travels by land with the destination Nicaragua. Since 2020, he uses a new route through the Ngobe bugle region and spent the night in a place at Buabidti. Once at his destination, remains in Managua and sometimes travels to Chinanderas. Physical examination was remarkable with wasting, painful splenomegaly (GIII) and hyperchromic nodular lesion in the forearm. CBC with

pancytopenia, elevated DHL, Lymphocyte TCd4+: 72 cells/mcl and HIV VL: 326, 622 copies/ml and Hypergammaglobulinemia. Abdominal CT scan revealed hepatosplenomegaly at expense of the spleen (approximately 15.5 cm) and scattered lymph nodes without necrosis. A biopsy was performed on the skin lesion which revealed the presence of *Leishmania spp.* amastigotes. 5mL of blood was collected from patient and was centrifuged at 2200g for 20 minutes to assure buffy coat separation. DNA was isolated from 200 µL buffy coat using High Pure PCR templates preparation kit (Roche, USA). A Taq man Real Time PCR assay targeting a conserved region of Leishmania REPL repeats (L42486.1) specific for *L. donovani* and *L. infantum* was used to detect DNA from *L. infantum*. Bone marrow analysis was negative as well as the kr39. Upon admission he received empiric therapy with deoxycholate Amphotericin B due to clinical suspicion of histoplasmosis and after improvement it was switched to itraconazole, however the symptoms relapsed. Because of the findings we restarted the AmphoB and observed clinical improvement. Antiretroviral therapy was initiated, and the patient was discharged. This patient, with evidence ACL meets the clinical criteria of Kala-Azar, characterized by fever, wasting, pancytopenia, hepatosplenomegaly and hypergammaglobulinemia. With confirmation of dissemination by PCR. The lack of isolation of *L. infantum* in the bone marrow may be because the patient was receiving amphotericin B since admission. Panama is endemic for cutaneous leishmaniasis, the main species being *L. (V.) panamensis, L. (V.) braziliensis and L. (V.) guyanensis,* so far, no cases of *L. infantum* have been described. Panama has the vector *Lutzomya longipalpis*, but in this case the cycle is unknown. It remains to be determined whether the presence of the vector is responsible for anthropozoonotic transmission. More studies are needed to determine the origin of this parasite as well as the presence of other cases in the region.

Keywords *Leishmania infantum*; PANAMA; NODULAR; VISCERALIZATION; KALA-AZAR

O7-06: MUCOCUTANEOUS LEISHMANIASIS DUE TO *L. (V) braziliensis*, A CHALLENGING THERAPEUTIC APPROACH. A CASE REPORT IN URABÁ, ANTIOQUIA

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Leishmaniasis is a parasitic disease transmitted by vectors, with clinical presentations in cutaneous, mucosal (or mucocutaneous) and visceral forms. The cutaneous form represents 98% of the cases in Colombia, while the mucosal and visceral form represents 1% of the cases. Mucocutaneous leishmaniasis (LM) is characterized by destructive compromise of the mucous membranes of the airway (nose, mouth, pharynx, larynx), conjunctiva, and genital area. Likewise, authentic LM is the one that occurs after a skin lesion, in an anatomical site distant from the affected mucosa. A case report is made of an 88-year-old male patient living in the rural area of Urabá, Antioquia, diagnosed with extensive cutaneous leishmaniasis in the right forearm, who presented a recurrence three years after receiving treatment with oral miltefosine in coadjuvant with *Artemisia annua* and topical extract of *Caesalpinia spinosa*. In the recurrence he presented extensive skin lesions, new lesions in the hand of the compromised extremity and in the face, as well as compromise of the nasal mucosa. Treatment with pentamidine isethionate (54 mg/kg accumulated) was prescribed, later arriving a report of L.(V.) braziliensis as the causal agent. Because a partial response was obtained with this medication and the symptoms reappeared in the nasal mucosa, treatment with amphotericin B (35 mg/kg accumulated) was performed, in coadjuvant with and topical management with *Caesalpinia spinosa* extract (Alyeyuba® lotion and emulsion) achieving a satisfactory clinical response. This case illustrates a

severe form of the disease, with difficulties in responding to treatment. In Colombia, the management of LM is standardized with antimonial derivatives, as the first line of choice, which were not considered in this patient due to his advanced age. In the initial treatment, miltefosine was chosen, since the species subsequently identified has been little reported in the region, with less efficacy for this species, explaining the recurrence of the lesion with the first systemic treatment. On the contrary, with pentamidine isethionate a cure rate of up to 100% is described for the species *L. (V.) braziliensis*, however, due to the poor clinical response, treatment was changed to liposomal amphotericin B, achieving clinical cure with a combination of systemic and topical drugs. Likewise, it contributes to the knowledge of the epidemiology of leishmaniasis in Urabá Antioquia and highlights the importance of identifying the *Leishmania* species causing the clinical picture, for the establishment of adequate treatment, as well as the importance of long-term follow-up of patients treated with cutaneous forms, so that the morbidity caused by the mucosal form of the disease can be prevented. Finally, this work shows difficulties in the treatment of complex manifestations of LC, which can be circumvented by combining conventional treatments with other therapeutic alternatives under development, both topical (Alyeyuba®) and oral (*Artemisia annua*), allowing the complete healing of skin lesions.

Keywords MUCOCUTANEOUS LEISHMANIASIS; *L. (V.) braziliensis*; RECURRENCE; PENTAMIDINE; AMPHOTERICIN B; CASE REPORT

O14-01: IMPROVING DIAGNOSIS OF CUTANEOUS LEISHMANISIS; MINIMALLY INVASIVE SAMPLING TOOLS, RAPID TESTS, AND SAMPLING LOCATION

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Cutaneous leishmaniasis (CL) is common in Ethiopia, mainly affecting impoverished populations in rural areas with poor access to health care. CL is routinely diagnosed using skin slit smear microscopy, which requires painful sample collection, skilled staff and appropriately equipped laboratories. We evaluated the CL Detect Rapid Test (InBios) and several less invasive sample collection tools to assess the value of alternative more patient-friendly diagnostic strategies which could be used in field settings. We conducted a cross-sectional study at the Leishmaniasis Research and Treatment Center in Gondar, Ethiopia among patients with suspected CL lesions. Samples taken were microbiopsy (a minimally invasive tool that mimics a sandfly bite, Trajan), tape disc (a sticky plastic disc placed on the lesion stripping the top layer of the skin, CuDerm), dental broach (a thin barbed needle inserted in and out of the lesion to retrieve tissue), and skin slit (scraping tissue from underneath skin after incision with a scalpel). Tests evaluated were microscopy, CL Detect Rapid Test, and (kinetoplast DNA) qPCR. First, we evaluated the diagnostic accuracy of the CL Detect Rapid Test on skin slit and dental broach samples against a combined reference test of microscopy and qPCR targeting kinetoplast DNA on the skin slit sample. All different sample collection tools were compared by qPCR, and sample collection from the border vs the center of the lesion will also be

compared by qPCR on microbiopsy samples. Recruitment of this study is still ongoing (current inclusion 258 patients); the latest available results will be presented. Interim analysis for the evaluation of the CL Detect Rapid Test on 165 patients showed that 128 (77.6%) patients had confirmed CL. Of these, all 71 microscopy-positive patients were also positive by skin slit PCR, and 57 patients were positive for skin slit PCR only. Sensitivity of the CL Detect Rapid Test on skin slit was 31.3% (95% confidence interval (95%CI) 23.9-39.7), which was significantly higher (p=0.010) than for the dental broach (22.7%, 95%CI 16.3-30.6). Sensitivity of the CL Detect Rapid Test for both sampling methods was significantly lower (p<0.001) than of routinely used microscopy, which had a sensitivity of 55.5% (95%CI 46.8-63.8) compared to skin slit PCR as a reference. Comparison of different sample collection methods on this population is ongoing, although an embedded pilot study on the use of microbiopsies showed that they are highly sensitive and are much less painful than skin slit samples. We found that the sensitivity of the CL Detect Rapid Test was low on skin slit as well as on dental broach samples, and performed poorer than microscopy. We therefore do not consider it suitable for routine use in Ethiopia. Minimally invasive sampling tools seem sensitive and well tolerated by patients, and may perform better than invasive methods. Results from this study will help to optimize diagnosis of CL.

Keywords *Leishmania aethiopica*; RAPID DIAGNOSTIC TESTS; POINT-OF-CARE TESTS; DIAGNOSTIC TESTS, REAL-TIME PCRc

O14-02: DEVELOPMENT OF ENHANCED SENSITIVITY TOOLS TO MONITOR *Leishmania* INFECTION

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Leishmaniases are neglected tropical diseases caused by an intracellular protozoan parasite transmitted by female phlebotomine sandflies. Each year this disease causes 30 000 deaths worldwide. Different forms exist, showing gradual severity : a cutaneous form conferred by *L. major*, a mucocutaneous form, and a visceral form conferred by *L. infantum*, which is fatal when left untreated. Today only few treatments are available, and they present issues of toxicity, high risks of relapse and emerging resistance. Therefore, development of new treatments is necessary, and requires models allowing to monitor *Leishmania* survival. We propose the creation of bioluminescent and fluorescent strains stably expressing enhanced bioluminescence coupled to fluorescence to facilitate screening of new compounds against *Leishmania,* and to avoid necessity of multiple cultures for *in vivo* and *in vitro* detection. Electroporation of different constructs were realized on two species of *Leishmania* : *L. major* and *L. infantum*. Selection of parasites allowed to isolate single clones stably expressing both luciferase and fluorescent protein. Follow-up of growth and mortality by flow cytometry indicated that insertions did not alterate parasite development. Both fluorescence and bioluminescence were stably expressed in transformed strains of *L. major* and *L. infantum,* and signals decreased as mortality increased. Bioluminescence emission showed a strong correlation (R^2=0,9) with parasite concentration. Finally, fluorescent parasites were observed inside primary murine macrophages with confocal microscopy, confirming parasites maintained their capacity to infect host cells. Current treatments present multiple drawbacks, leading to the urgent

need of new alternatives, which implies to set up new tools to perform efficient and fast screenings of drug libraries. These new reporter strains are a tool for rapid screening of anti-leishmanial compounds, and the possibility to use *L. major* and *L. infantum* presents an opportunity to rapidly screen for broad-spectrum anti-leishmanial compounds.

Keywords LEISHMANIASIS; REPORTER STRAINS; BIOLUMINESCENCE

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O14-03: ELISA WITH Lb6H RECOMBINANT ANTIGEN VALIDATED FOR DIAGNOSING AMERICAN CUTANEOUS LEISHMANIASIS

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American cutaneous leishmaniasis (ACL) has a wide distribution, with an annual incidence of between 600,000 and 1 million new cases, mainly in tropical and subtropical countries. In Brazil, in 2020, 16,432 cases of cutaneous and mucocutaneous leishmaniasis were reported. The diagnosis of ACL is based on an Epidemiology-Clinical-Laboratory tripod. The clinical-epidemiological aspects are fundamental, but they need laboratory exam data to confirm the diagnosis, mainly due to the varied clinical aspects that are not pathognomonic. The Montenegro test was an important support in diagnosing ACL. However, its antigen production was discontinued leaving

a gap in the diagnostic procedure, particularly in resource-poor settings. There is no single gold standard diagnostic test; thus, laboratory assays that suggest Leishmania infection are needed. We had previously obtained excellent performance of *Leishmania (Viannia) braziliensis*-derived gene sequence, the Lb6H recombinant antigen (rLb6H) in an ELISA platform for the diagnosis of ACL caused by *L. braziliensis*, *L. amazonensis,* and *L. guyanensis*. The present study aimed to validate rLb6H-ELISA for potential use in ACL diagnosis. We analyzed four panels containing 1.091 samples from leishmaniasis patients and healthy controls living in various Brazilian endemic and non-endemic localities. In the reference panel (panel 1), composed of 70 samples from patients with cutaneous and mucosal leishmaniases and 70 healthy controls, the ELISA-rLb6H showed a sensitivity of 98.6% (95%CI: 92.3-99.9) and a specificity of 100.0% (95%CI: 94.8-100.0). In addition, reproducibility was evaluated, obtaining a coefficient of variation of positive samples ≤ 8.20% for repeatability, ≤ 17,97% for reproducibility, and ≤ 8.12% for homogeneity. Then, the stability of the antigen on the plate with time was evaluated. The plates sensitized with the recombinant antigen were stable at 4°C and -20°C for 180 days, and the accelerated stability study (37°C) indicated the validity of 12 months without loss of reactivity. In samples of patients with ACL from five research and health care centers in endemic and non-endemic areas (panel 2), the standardized assay showed a sensitivity of 84.0% (95%CI: 80.0-87.3); no significant statistical difference was observed among the five centers (chi-square test, p=0.13). In samples of healthy controls from four areas with different endemicity (panel 3), a specificity of 92.4% (95%CI: 89.2-94.7) was obtained; lower specificity was obtained in a locality presenting high endemicity for visceral leishmaniasis (chi-square test, p<0.001). In the interference panel to assess the cross-reactivity with ten diseases (panel 4), positive results were obtained in 13.9% (95%CI: 9.3-20.0) of samples, with high positivity for tuberculosis, malaria, and paracoccidioidomycosis. Based on the good diagnostic performance and the reproducibility and stability of the antigen, we suggest using ELISA-rLb6H to diagnose ACL.

Keywords SEROLOGICAL TESTS; ELISA; CUTANEOUS AND MUCOCUTANEOUS LEISHMANIASES; RECOMBINANT PROTEINS

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O14-04: CHARACTERIZATION OF NEW WORLD CUTANEOUS LEISHMANIASIS LESIONS USING NOVEL, NON-INVASIVE METHODS

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New World cutaneous leishmaniasis (NWCL) is a debilitating and disfiguring parasitic disease that predominately manifests as ulcerative dermal lesions. However, there is a limited pharmacological understanding of the lesion site. As the biomechanical and biophysical characteristics of skin describe its integrity and permeability, this study sought to measure properties commonly correlated with skin barrier composition and function in NWCL lesions including transepidermal water loss (TEWL), hydration, sebum, pH, surface temperature, elasticity (Young's modulus, viscoelasticity, and retraction time), echostructure (collagen intensity and low echogenic band), CIELAB color, and skin texture. Thirty localized, uncomplicated NWCL lesions from 23 Colombian patients having parasitologically-confirmed cutaneous leishmaniasis were prospectively characterized prior to treatment and during lesion healing using a non-invasive, *in situ* dermal analyzer system (DermaLab Combo 2.0) and various imaging techniques (ultrasound, videoscope, dermascope, 3-D, and smartphone). Measurements and images were collected from both affected skin (lesion center and lesion border) and unaffected skin (peri-lesion and contralateral limb) at 0, 10, 25, and 60 days from treatment initiation. At three months following completion of treatment, patients were contacted to confirm therapeutic outcome. NWCL lesions demonstrated considerable structural and functional skin barrier abnormalities. Of note, skin barrier properties of NWCL lesions experience clinically-significant physiological changes in integrity compared to uninvolved skin, chiefly a ten-fold initial increase in TEWL and twenty-fold initial increase in hydration, demonstrating greater permeability. These data suggest altered dermal

pharmacokinetics and pharmacodynamics (PK/PD) at the site of local infection. For all parameters, this structural and functional disruption is particularly discernable prior to treatment administration. Moreover, treatment-initiated recovery of the skin barrier defect is delayed and gradual. The diminished integrity of lesional skin is largely maintained during treatment and can persist following completion of treatment, most clearly demonstrated by the lesions' TEWL, elastic properties, and echostructure. Broadly, this study establishes a basis for understanding the altered skin physiology and skin barrier function at the dermal lesion site, both due to NWCL infection and as a consequence of the treatment-initiated healing process. This work can inform the development of both local drug delivery systems for NWCL and the design of physiologically-relevant *in silico* and 3-D human skin equivalent models that recapitulate the NWCL lesion environment *ex vivo*. The development of such models from these patient data could have broad implications for enhanced topical and transdermal formulation testing. The utility of these non-invasive characterization techniques, chiefly TEWL and hydration, for improved monitoring of the therapeutic response or for prediction of treatment failure is promising but warrants explicit exploration in a larger cohort.

Keywords SKIN PHYSIOLOGY; SKIN PERMEABILITY; WOUND HEALING; CUTANEOUS LEISHMANIASIS

O14-05: TRAINING AND PERFORMING EVALUATION FOR PARASITOLOGICAL DIAGNOSTIC OF LEISHMANIASIS IN DIGITAL FORMAT: AN EQUITABLE PERFORMANCE PROPOSAL

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The cutaneous leishmaniasis (CL) diagnosis is based on clinical suspicious and confirmed by laboratory exams. The most used laboratory method, mainly for CL, is the direct parasitological (DP) examination, useful to diagnose around 80% (31,697) of the total cases reported in the Americas in 2020. The DP consists of visualizing the parasite by microscopic examination of stained slides containing smears collected by scarification or biopsy of the lesion. However, the test accuracy depends on several factors, including the experience of the microscopist. In turn, an accurate diagnosis results in access to adequate treatment, ensuring the efficiency of the health service. Based on these assumptions, PAHO has been applying since 2015 the Direct External Performance Evaluation Program (PEED, of the Spanish 'Programa de Evaluación Externa del Desempeño', which aims to harmonize the procedures for the microscopic diagnosis of cutaneous leishmaniasis and to evaluate the performance of the reference national laboratories in the American countries with CL transmission. So far, the PEED strategy has been

based on a 10 slides panel using different samples collected from experimentally infected animals. The panel is prepared to be representative of the diversity observed in the real life of a laboratory, either in parasitism or in the quality of the slide. In 2021, a Digital PEED version was proposed aiming to improve the standardization of the quality assessment process and to make it the most reliable of the clinical diagnosis reality. All the labs were challenged by the same panel through a digital tool for scanning slides, prepared from lesions of patients with clinical suspicion of CL. The slides were scanned in a Metafer Scanning and Imaging Platform and then, projected onto a computer screen using MetaSystems software. The scanned slides can be visualized in a high-resolution mode, in a simulation of the manual microscopic examination. The validation of the process was conducted with three reference laboratories from Brazil, all of them previously submitted to the PEED assessment using the microscope reading strategy. The performance of the reference laboratories, defined as the number of correct results over total evaluated slides, was high (9/10, 9/10, and 8/10), confirming the viability of the digital assessment strategy. This first experience identified the need to improve the image download system to speed up the process. The digital PEED has the advantage of making the same panel of 10 slides available to all laboratories participating in the program, allowing direct comparison of results. In conclusion, the digital PEED emerge as a promising modality of quality assessment for the parasitological diagnosis, with the potential to be expanded, to reduce costs and to produce equity in performance comparison, improving the CL diagnosis in the Americas.

Keywords CUTANEOUS LEISHMANIASIS; MICROSCOPIC EXAMINATION; PARASITE VISUALIZATION; PEED

Financing Coordenação de Vigilância em Saúde e Laboratórios de Referência (CVSLR) - Fiocruz; Pan American Health Organization - PAHO/OMS

O21-01: DEVELOPMENT AND ASSESSMENT OF *Leishmania major* AND *Leishmania tropica* SPECIFIC LOOP-MEDIATED-ISOTHERMAL AMPLIFICATION ASSAYS FOR THE DIAGNOSIS OF CUTANEOUS LEISHMANIASIS IN TUNISIA

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Cutaneous leishmaniasis (CL) remains one of the world's most prevalent neglected diseases, particularly in developing countries. Identification of the involved *Leishmania* species is an important step in the diagnosis and case management process. In this study, we tested simple, rapid, and highly sensitive loop-mediated isothermal amplification (LAMP) assays for *Leishmania* DNA species-specific detection from cutaneous lesions. Two LAMP assays, targeting cysteine protease B (cpb) gene, were developed to detect and identify *Leishmania major* and *Leishmania tropica* species. Loop-mediated isothermal amplification specificity was examined using DNA samples from other *Leishmania* species and *Trypanosoma* species. No cross-reactions were detected. The developed LAMP assays exhibited sensitivity with a detection limit of 20 fg and 200 fg for *L. major* and *L. tropica*, respectively. Both tests were applied on clinical samples of CL suspected patients living in endemic Tunisian regions and compared with kinetoplast DNA quantitative PCR (qPCR), microscopic, and conventional cpb-based polymerase chain reaction (PCR) assays. Our LAMP tests were able to discriminate between *L. major* and *L. tropica* species and showed a sensitivity of 84% and a specificity of 100%. However, when compared with the performance of the diagnostic tests with latent class analysis (LCA), our

LAMP assays show a sensitivity of 100%. These assays can be used as a first-line molecular test for early diagnosis and prompt management of CL cases in public health programs.

Keywords LAMP; CPB; DIAGNOSIS; PCR; RT-PCR

O21-02: APTAMERS FOR THE DIAGNOSIS OF LEISHMANIASIS

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Leishmaniasis is a vector-borne tropical disease that, depending on the causative species, causes 3 different clinical manifestations: cutaneous, mucosal, and visceral leishmaniasis. In Colombia, *Leishmania (viannia) panamensis* is responsible for up to 79% of cases. Leishmaniasis diagnosis lacks a gold standard method, and the most sensitive tests can only be performed in laboratories with specialized equipment to perform qPCR, ELISA, and IIF. Accordingly, synthetic molecules such as aptamers have shown the potential to play a role in the development of accurate biosensors for the diagnosis and monitoring of leishmaniasis in endemic areas. In this work, sera samples from patients infected with cutaneous and mucosal leishmaniasis were used to select DNA aptamers that recognize a previously identified protein which was named *Leishmania panamensis* D protein (LpD) as a biomarker for mucosal leishmaniasis through Systematic Evolution of Ligands by Exponential Enrichment (SELEX). We present a list of unique NGS-identified aptamers with biomarker recognition for cutaneous and mucosal leishmaniasis evaluated by both ELASA and western blot assays. We propose that the interaction between PD1 aptamer and LpD recombinant protein is mediated by two hydrogen bonds. One of these bonds involves the Glu119 base and the G50 base which takes part in the predicted motif for LpD protein recognition. The aptamers PD1, PD2 and PD3 showed a low dissociation constant (Kd) value 51.83±8.725nM, 63.38±9.863nM and 84.85±15.87nM respectively, which makes them good specific biosensors candidates that will allow rapid and low-cost diagnosis of leishmaniasis in remote and low-resource endemic areas. In addition, we consider that a portable tool could be used in the field to further improve the sensitivity and specificity of this type of diagnostic test.

Keywords APTAMERS; LEISHMANIA; DIAGNOSIS; WESTERN BLOT; ELASA

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O21-03: DIAGNOSTIC TESTS ACCURACY USED IN THE TEGUMENTARY LEISHMANIASIS: A CROSS-SECTIONAL ANALYSIS IN THE ROUTINE AT THE BRASÍLIA UNIVERSITY HOSPITAL

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American Tegumentary Leishmaniasis (ATL) has in Brazil, the highest incidence in the Americas, being a public health problem in this country. ATL needs an accurate diagnostic method because the medicines used in its treatment present significant and serious adverse effects. The gold standard diagnostic exam, the Polymerase Chain Reaction (PCR), is still a high cost exam in Brazil, and its routine use is not feasible in public hospitals. For this reason, combined parasitological and non-parasitological diagnostic methods are used in reference centers in order to achieve greater accuracy. This study aimed to perform the cross-sectional analysis of the diagnostic accuracy, sensitivity and specificity of those tests performed in the Leishmaniasis routine diagnostic in the dermatology outpatient clinic of Brasilia University Hospital (HUB), a reference center specialized in ATL, a government hospital, free of charge for the general population. For this study, were analyzed the following diagnostic tests: culture, skin biopsy smear, indirect Immunofluorescence (IFI), intradermoreation of Montenegro (IDRM) and histology (HT) which had their results analyzed individually and in pairs. This study included 197 patients, divided into groups of cases (157 patients) and controls (41 non-patients). To be included in the patient group, they should present suggestive symptoms associated to a positive test(s) results: one parasitological or two non-parasitological was required. Patients under immunosuppression

conditions, as well as special populations (indigenous, pregnant and under 18 years) were excluded from the study. Clinical data were obtained from hospital and Dermatomicology laboratory records. The statistical analysis was obtained by the Openepi platform, using a confidence interval (CI) of 95% and the probability of significance less than 5% ($p<0.05$). In the individual study, the tests with the highest sensitivity were the IDRM and the HT (89.92% and 90.54%, respectively), and the parasitological tests (culture and smear) were the ones that reached the highest specificity (100%). Regarding accuracy, HP and IDRM reached the highest indices: 84.95 and 83.54%, respectively. In peer analysis, the HP+IFI and HP+ culture association, achieved higher accuracy (86.87%), followed by HP+ smear (86.36%), IDRM+ HP (84.26%), IDRM+ culture (81.73%), IDRM+ smear (81.41%), IDRM+ IFI (80.71%), IFI + smear (79.29%), IFI + culture (76.65%) and culture + smear (59.09%). Since accuracy is the probability of a diagnostic test providing correct results, being positive in patients and negative in non-patients, the test with the best combination of sensitivity and specificity will be the most accurate. Thus, the peer association strategy was the one that achieved better accuracy by including an examination with high sensitivity and another with high specificity. However, considering the cost, time and practicality, the combination of IDRM + smear would present a better cost benefit, because in addition to high accuracy (81.41%), it has a fast and lower cost result.

Keywords AMERICAN TEGUMENTARY LEISHMANIASIS; DIAGNOSIS; PARASITOLOGICAL EXAMS; NON-PARASITOLOGICAL EXAMS

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O21-05: THE ADDED VALUE OF PCR FOR DETECTION OF *Leishmania donovani* FROM MICROSCOPY NEGATIVE TISSUE SMEARS OF SUSPECTED PATIENTS IN GONDAR, ETHIOPIA

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Visceral leishmaniasis (VL) is a major public health problem in Ethiopia and is fatal if left untreated. Therefore, reliable diagnostics are pivotal for clinicians to decide which patients should receive antileishmanial treatment. Routinely, microscopic examination of spleen or bone marrow aspirates is done to guide clinical decision making, but this method lacks sensitivity. Molecular tests are more sensitive and considered most accurate to diagnose patients. However, PCR is quite costly wherefore it is not routinely used in low-resource settings, and solid evidence on the benefits of PCR over microscopy for diagnosis of VL is lacking. In this study, we evaluated the added value of real-time PCR for detection of *Leishmania donovani* on microscopically negative tissue slides. A retrospective study was performed on a sample of stored microscopically negative (*n*=193) spleen and bone marrow smears from primary VL suspected patients, relapse cases and discharged patients (test-of-cure). The slides were collected at the Leishmaniasis Research and Treatment Center (LRTC) of the University of Gondar, northern Ethiopia between June 2019 and November 2020. Sociodemographic, clinical and treatment data were recorded, and DNA was isolated from the tissue slides and tested for the presence of *Leishmania* kinetoplast DNA by real-time PCR. Among the microscopy negative slides, 62.3% (95%CI 55.0 - 69.1) was positive by PCR with a median Ct of 33.0 SD±6.5, and PCR had a significant added value for VL diagnosis compared to microscopy (p<0.001). PCR identified a significant

number of additional positives for both microscopically negative spleen (54.1%) and bone marrow (82.1%) samples, although the PCR positivity rate was much higher for the latter. Despite the rK39 rapid diagnostic test (RDT) confirmation of all microscopy positive, and 72.0% of the microscopy negative but PCR positive primary VL patients, 59.2% of the PCR negative patients were also rK39 positive. Overall, 57.4% (95%CI 48.4 - 65.9) of the patients with a PCR positive result did not receive antileishmanial treatment. Similarly, the added value of PCR to identify patients that need treatment was larger ($p<0.001$) for bone marrow (80.6%, 95%CI 61.9 - 91.9) than spleen (50.0%, 95% CI 40.3 - 59.7) aspirates. Our study shows that PCR leads to a substantial number of additional positive patients for microscopy negative suspected VL patients and that there is likely considerable undertreatment of VL patients in northern Ethiopia. Therefore, we recommend PCR as a second-line diagnostic method in microscopy negative patients. Microscopy slides can be sent to referral centers where molecular facilities are available, without the need of taking an additional sample. If resources are limited, one should focus on suspected primary VL patients with a microscopy negative bone marrow sample. In contrast, results of the rK39 RDT should be interpreted with care, as it could result in both over- and undertreatment of patients.

Keywords VISCERAL LEISHMANIASIS; MICROSCOPY; PCR; TREATMENT; DIAGNOSTICS

O21-06: ANTIGEN TESTS FOR *Leishmania* INFECTION

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Visceral leishmaniasis (VL) is being targeted for elimination on the Indian subcontinent (ISC). Asymptomatic *Leishmania* infections (ALI) outnumber clinical infections. Furthermore, people living with human immunodeficiency virus (PLHIV) have a higher risk of developing VL with poorer outcomes in comparison to immunocompetent individuals. ALI and *Leishmania*-HIV coinfection present a challenge for VL elimination and clinical management. As India moves towards VL elimination, diagnostics which enable early detection of infection may be essential to eliminate reservoirs and improve clinical outcomes. These data form part of larger studies: 1. A cross-sectional survey of 720 individuals from September 2016-March 2018 living in VL-endemic regions of Bangladesh, with no symptoms or history of VL and post kala-azar dermal leishmaniasis (PKDL), and who were a contact of an individual with VL or PKDL. ALI was detected by quantitative polymerase chain reaction (qPCR), the direct agglutination test (DAT), loop-mediated isothermal amplification (LAMP), and/or the *Leishmania* Antigen enzyme-linked immunosorbent assay (ELISA) (Clin-Tech, UK, formerly Kalon Biological, UK) which measures *Leishmania* antigen excreted in urine and therefore non-invasively detects current infection. Work was carried out in partnership with the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) and the Foundation for Innovative New Diagnostics (FIND); 2. A Médecins Sans Frontières (MSF)-led non-interventional cross-sectional survey and prospective 18-month follow-up of 1,300 PLHIV with no history of VL or PKDL from May 2018-November 2020 presenting at antiretroviral therapy clinics in VL-endemic areas of Bihar, India (CTRI/2017/03/008120). ALI was detected by qPCR, rK39 ELISA, and/or rK39 rapid diagnostic test (RDT), with the *Leishmania* Antigen ELISA run but not included in the primary definition of ALI. In Bangladesh, 69 (9.6%) participants had ALI detected by a combination of *Leishmania* antigen ELISA (3.3%), qPCR (1.0%), LAMP

(2.1%), and DAT (3.9%), with one (0.1%) participant positive by four tests. All individuals positive by more than one test were detected by the *Leishmania* antigen ELISA and DAT in combination. In India, 96 (7.4%) PLHIV had ALI detected by a combination of rK39 ELISA (100%), rK39 RDT (0.4%), and qPCR (0.5%). Twenty-eight (2.2%) participants were positive by the *Leishmania* antigen ELISA, 20 (1.5%) of whom were in addition to the primary definition of ALI. In India, the ALI and non-ALI cohorts were followed up, with four (3.7%) participants progressing from ALI to VL only, all four of whom were *Leishmania* antigen and rK39 ELISA positive at baseline. Median urinary antigen was 1932.0 UAU/ml at baseline in the four individuals who developed VL, compared to 12.4 UAU/ml in individuals with ALI who did not develop VL during follow-up. Three (75.0%) of four participants with ALI with matched urine samples at baseline and 18-months remained positive for the *Leishmania* antigen ELISA. Antigen testing may be of benefit for monitoring transmission and surveillance of *Leishmania* infection in combination with serology, where early detection of ALI including in PLHIV may improve patient management and reduce the burden of disease in an elimination setting. The development of an RDT to detect *Leishmania* antigen would further benefit the elimination campaign.

Keywords *Leishmania*; ANTIGEN; DIAGNOSTICS; ASYMPTOMATIC; VL

O28-01: *IN VITRO* AND *IN VIVO* COMBINATION PROFILING FOR LEISHMANIASIS TREATMENT USING A BACK-TRANSLATIONAL APPROACH

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Leishmaniasis, a parasitic tropical disease caused by *Leishmania* spp. and transmitted by female sandflies, is strongly associated with poverty, affecting mainly neglected populations with poor access to good health care systems. Visceral leishmaniasis (VL) is the most severe form of the disease, fatal if left untreated and endemic in three main global regions (South Asia, Eastern Africa and Latin America). Existing treatments for VL are based on five drugs, namely sodium stibogluconate, meglumine antimoniate, paromomycin, liposomal amphotericin B and miltefosine. All have severe limitations, such as toxicity, painful parenteral administration and/or the need for a cold-chain. The most recent and only currently available oral drug, miltefosine, still has poor gastro-intestinal tolerability and potential teratogenicity. Furthermore, varying levels of efficacy have been observed for these recommended treatments in the different endemic regions, both when used in monotherapy and in combination. DND*i* together with

academic and pharmaceutical partners is developing an unprecedented portfolio of new chemical entities (NCEs). It is expected that from among these, simple, safer, and orally active therapies (drug combinations or monotherapies) will emerge that can transform leishmaniasis management in all endemic areas, with straightforward patient administration integrated into primary health care settings. For the successful development of these NCEs, benchmarking and back-translational information from marketed drugs is missing and is urgently required to enable proper interpretation of *in vitro* and *in vivo* preclinical data, compared with information from patients in the field. In this study, we generated *in vitro* and *in vivo* data from several animal models of infection (an acute Balb/c mouse model and a chronic golden hamster model) for the two *Leishmania* spp. causing VL; *Leishmania donovani* and *Leishmania infantum*. *In vitro* and *in vivo* combination data for the currently recommended leishmaniasis drugs will be presented and compared with that of the new drug candidates. Efficacy and pharmacokinetic data from animal models will be presented and, together with previously collected efficacy and pharmacokinetic data from past clinical trials in VL patients, a translational relationship between laboratory and field data will be proposed. Furthermore, a similar approach is being applied to the development of new treatments for cutaneous leishmaniasis. These results are expected to have a significant impact on the drug discovery and translational process for finding new therapies to combat leishmaniasis, whilst simultaneously speeding up NCE development, enabling safer and more effective treatments for patients with VL or CL, as rapidly as possible.

Keywords LEISHMANIASIS; DRUG COMBINATIONS; NEW CHEMICAL ENTITIES; ANIMAL MODELS, PHARMACOKINETICS

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O28-02: MULTI-TARGET EVALUATION OF WIDELY USED PLANTS TO UNDERSTAND MEDICINAL PRACTICES IN THE TREATMENT OF LEISHMANIASIS ACROSS AMAZONIA

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Leishmaniasis are widely distributed worldwide among tropical and subtropical countries, especially in the Amazonian region. Indigenous groups across Amazonia have developed abundant knowledge about medicinal plants related to this pathology and its various clinical forms. Several reviews of knowledge and practices related to medicinal plants highlight cross-uses of plants among various cultural groups. A focus on

medicinal plants used in French Guiana by local population (Amerindian, Creole, Bushi-nengue) will be presented together with a recent study of the basis of medicinal practice in leishmaniasis treatment across Amazonia. In this study, we aimed to unravel the process of taxa selection for medicinal use in Amazonian communities. We assumed that specific activity of plant relies on several factors (anti-inflammatory, wound healing, immunomodulating, antimicrobial) besides leishmanicidal activity. The twelve most widespread plant species used against leishmaniasis in Amazonia, according to their cultural and biogeographical importance determined through a wide bibliographical survey (475 use reports), were selected for this study. Plant extracts were prepared to mimic their traditional preparations (fresh material; manual crushing; pool of acidic water extract, ethanol and ethyl acetate - extracts). Antiparasitic activity was evaluated against promastigotes of reference and clinical strains of *Leishmania* (*L. guyanensis, L. braziliensis* and *L. amazonensis*) and *L. amazonensis* intracellular amastigotes. We concurrently assessed the extracts immunomodulatory properties on PHA-stimulated human PBMCs and macrophage-like cells, and on *L. guyanensis* antigens-stimulated PBMCs obtained from *Leishmania*-infected patients. Antifungal activity and wound healing properties (human keratinocyte migration assay) of the selected extracts were also evaluated. The cytotoxicity of the extracts against various cell lines was also measured. Only *Spondias mombin* L. bark and *Anacardium occidentale* L. stem and leaves extracts displayed high anti-promastigotes activity, with IC50 ≤ 32 µg/mL against *L. guyanensis* promastigotes for *S. mombin* and IC50 of 67 and 47 µg/mL against *L. braziliensis* and *L. guyanensis* promastigotes, respectively, for *A. occidentale*. Anti-leishmanial activities of the twelve plant extracts were strain and stage dependent. Antifungal activity measured against *Candida albicans* and *Trichophyton rubrum* (MIC in the 16–64 µg/mL range) was also evaluated. However, in the case of *Leishmania* amastigotes, the most active species were *Bixa orellana* L. (seeds), *Chelonantus alatus* (Aubl.) Pulle (leaves), *Jacaranda copaia* (Aubl.) D. Don. (leaves) and *Plantago major* L. (leaves) with IC50 < 20 µg/mL and infection rates of 14–25% compared to the control. Concerning immunomodulatory activity, *P. major* and *B. orellana* were the most potent species for the wider range of cytokines in all tested conditions despite overall contrasting results depending on the model. Most of the species led

to moderate to low cytotoxic extracts except for *Chelonantus alatus*. None of the tested extracts displayed wound healing properties. We highlighted pharmacologically active extracts either on the parasite or on associated pathophysiological aspects, thus supporting the hypothesis that antiparasitic activities are not the only biological factor useful for antileishmanial evaluation. Plant cultural importance, ecological status and availability will be discussed in relation with biological results, in order to link ethnobotany, medical anthropology and biology.

Keywords *Leishmania*; MEDICINAL PLANTS; PLANT USE PATTERN; IMMUNOMODULATION; FRENCH GUIANA

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O28-03: TARGETING THE EXOPROTEOME OF PARASITES FOR DRUG THERAPY: *Leishmania* CASEIN KINASE 1 AS AN EXAMPLE

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A common feature of the most successful intracellular pathogens is the efficient manipulation of their host cell. The manipulation of macrophage by *Leishmania* occurs mostly by exporting proteins into the host cell. While most of the current drugs as well as those in development target processes in the parasite itself, targeting the exo-proteome of parasites, and particularly excreted signalling kinases, is an interesting unexplored option that may provide several advantages: it may restore the host cell ability to fight the parasite and limit the risk of parasite resistance. As a signalling kinase released in the macrophage via extracellular vesicles (EVs), *Leishmania* Casein Kinase 1 (L-CK1.2) is a very good candidate to develop such a strategy. L-CK1.2 is closely related to human CK1 and essential for intracellular parasite survival. The identification of L-CK1.2 binding partners in the parasite revealed its involvement in many processes such as protein transport, trafficking or post-translational modifications, confirming its pleiotropic nature. The secretion of L-CK1.2 via EVs, suggests it has a role in the host cell; and indeed, several evidences supports the hypothesis that it has been evolutionary selected to interact with and phosphorylate host proteins. Here, combining phosphatase treatment, *in*

vitro kinase assay, immuno-precipitation and quantitative mass spectrometry, we established the host interactome of L-CK1.2 and developed a method to identify its host substrates *in vitro*. Our results suggest that L-CK1.2 might regulate specific pathways in the host cell such as trafficking, apoptosis and translation; most pathways also modulated during *Leishmania* infection. Collectively, our data point to a key role for L-CK1.2 in host-parasite interactions and as such it represents an interesting target to identify novel anti-*Leishmania* drug therapies. After validating L-CK1.2 as a drug target for antileishmanial therapy, we then developed a pipeline from drug screening to target deconvolution to discover novel hit compounds targeting L-CK1.2. Several chemical series have emerged as potent antileishmanial hit compounds using this pipeline, including CTN1122. The TEXLEISH consortium is focused on developing this hit into an optimised lead compound. CTN1122 is a potent inhibitor of recombinant L-CK1.2 with a very good selectivity index towards mammalian MRC5 and RAW 264.7 cells (52.5 for *L. major* and 15.3 for *L. donovani*). CTN1122 is lethal for promastigotes as well as for intracellular and axenic amastigotes. Furthermore, using an antisense approach, we demonstrated that down-regulation of L-CK1.2 decreases the IC_{50} of CTN1122. When tested *in vivo*, it reduces the parasite load in the liver and spleen of mice infected with *L. donovani* as well as in the lesion of mice infected with *L. major* with a significant decrease in the size of the lesion. In conclusion, our results provide the first insights into the functions of L-CK1.2 in the macrophage and the first evidence that targeting the exoproteome for drug treatment provides fertile ground to identify potent antileishmanial compounds, which might limit the risk of selecting for drug resistant parasites.

Keywords CASEIN KINASE 1; EXOPROTEOME; DRUG TARGET; CELL SIGNALLING; ANTILEISHMANIAL DRUG THERAPY

Financing PTR539, TranSig-ANR-13-ISV3-000, ANR-11-LABX-0024-PARAFRAP, TEXLEISH-ANR-21-CE18-0026

O28-04: ALTERATION OF THE CELLULAR MECHANISM AND GENE EXPRESSION PROFILE OF *Leishmania (leishmania) infantum* TREATED WITH ANTIHISTAMINE DRUGS

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Visceral leishmaniasis is a systemic infectious disease caused by some protozoan parasites of the genus *Leishmania*, endemic in tropical and subtropical countries. The therapeutic arsenal is restricted and due to toxicity, prolonged administration, resistance, and high cost, there is an urgent need to identify and develop new drugs. Therefore, drug repositioning is a promising strategy for the discovery of new drugs through the investigation of a new therapeutic indication for a drug already available on the market. In previous studies, we demonstrated the activity against *Leishmania* (*Leishmania*) *infantum* of histamine H1 receptor antagonists, such as cinnarizine (CNZ), and cyproheptadine (CPH) and meclizine (MCZ). Thus, this work aimed to identify cellular alterations and molecular markers, through a panel of genes that code for proteins with different cellular functions, related to the activity response. For this, the 50% effective concentration (EC50) was determined after two hours of treatment in *L. infantum* promastigotes. Afterwards, alkalinization of acidocalcisomes and intracellular concentration of ATP were analyzed by fluorimetric assays, cellular ultrastructures by transmission electron microscopy and gene expression profile by RT-qPCR. The CNZ, CPH and MCZ drugs showed antileishmania activity against *L. infantum* promastigotes with EC50 values from 8 to 45 µM. These drugs induced in promastigotes alkalinization of acidocalcisomes and reduced ATP levels, causing cellular damage associated with the bioenergetic system. The alpha-tubulin gene was identified as a reference gene for the normalization of gene expression, allowing analysis alteration in the gene expression profile of 11 genes with functions related to ATP synthesis; mRNA translation and stability; antioxidant defence;

transport membrane; carbohydrate metabolism; lipid metabolism and protein conformation and stability. These data, besides presenting direct results for the treatment of leishmaniasis, with the development of new alternatives, present great potential as a system for evaluating the activity response of various types of drugs.

Keywords VISCERAL LEISHMANIASIS; DRUG REPOSITIONING; ANTIHISTAMINE; ELECTRON MICROSCOPY; GENE EXPRESSION.

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028-05: BIOPHYSICAL AND FUNCTIONAL VALIDATION OF NEW PUTATIVE INHIBITOR OF AKT-LIKE OF *Trypanosoma cruzi*: A NOVEL THERAPEUTIC ALTERNATIVE FOR CHAGAS DISEASE

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Structural characterization and mechanism-of-action studies of therapeutic targets play relevant roles in the discovery of drugs that selectively modulate their cellular functions. The current need for safer and more effective treatments for tropical diseases has prompted the continuous search for enzymes as drug targets. The specific similarities of parasite kinases to their human homologs, which have been repeatedly shown to be druggable, as well as key structural, functional, and cellular context differences, make kinases attractive candidate targets for antiparasitic. The study at the cellular, molecular, and structural levels of the kinase AKT-like (EC 2.7.1.37) from *Trypanosoma cruzi* has generated results that support its potential as a pharmacological target for Chagas disease treatment. Our group found a putative inhibitor of AKT-like (UBMC4, patent No. 45984-Colombia) that causes loss of mitochondrial membrane potential (>90%), changes in morphology, and low percentages of cells with hypodiploidy, a mechanism of death associated with the apoptosis-like process. For the development of new strategies against Chagas disease, we propose the biochemical study of the enzyme AKT-like and biophysical studies of a specific inhibitor. In this study, the TcAKT-like protein and its pleckstrin domain were built, expressed, and purified employing recombinant DNA technology. Proteins purifications were performed by FPLC using an ÄKTA System via a nickel-affinity column and size-exclusion chromatography. Polyclonal antibodies in rabbits were raised against the full-length recombinant protein and used in immunodetection assays of endogenous parasite protein. The kinase activity of the recombinant was measured using

based on a solid phase enzyme-linked immunosorbent assay, and its melting temperature was determined using a fluorescence-based assay. Binding interaction between TcAKT-6His and UBMC4 ligand was determined by Saturation-Transfer Difference (STD) NMR and ^{15}N-heteronuclear single quantum correlation (HSQC). NMR measurements were recorded on a Bruker Avance III 700 MHz spectrometer equipped with a cryogenically cooled 5 mm TCI probe using z-axis gradients at 298 K. We expressed and purified the pleckstrin domain and full-length active AKT kinase of *T. cruzi* in *E. coli*. The concentration of proteins and the purity were sufficient for the biophysical analysis. The 1D-^{1}H NMR spectra of UBMC4 showed sharp peaks (5,5 – 9 ppm corresponding to Ar-H interaction); however, upon binding to protein TcAKT-6His, broadening of the peaks and subsequently decrease in the ligand's NMR signal height are observed due to the formation of the ligand-protein complex. the N-terminal PH domain was labeled with 15N and studied by 15N-HSQC spectra. When the ligand UBMC4 was added changes in the chemical shift were not observed during titration. Thus, although UBMC4 interacts with the full-length protein, we found that it does not interact with the isolated amino-terminal domain. The results of this work showed the first experimental evidence of the interaction between the TcAKT-like enzyme and the inhibitor UBMC4 by NMR. We applied biophysical and biochemical techniques in the study of the structural features of the ligand-target interaction in the frame of a rational drug design initiative toward the development of new trypanocidal agents.

Keywords CHAGAS DISEASE; AKT/PKB; INHIBITOR; DRUG TARGET; KINASE

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O28-06: INDUCTION OF ULTRASTRUCTURAL AND PHYSIOLOGICAL CHANGES IN MACROPHAGES AND PARASITES BY ANTILEISHMANIAL TRITERPENOIDS

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The current of antileishmanial treatment has proved the need for new therapeutic alternatives because the adverse effects and parenteral administration of the drugs result in the abandonment of treatment by the patients and the rise of leishmaniasis incidence in tropical regions. One of the goals for leishmaniasis control is to try to find, safe and efficacy drugs. For these reasons, natural compounds like triterpenes had become a source of antiparasitic drugs. However, the variability batch to batch and low extraction yield compromises the biological activity. According to the above, synthetic compounds can accomplish the accuracy and homogeneity to become antileishmanial drugs. In Our work, we evaluated synthetic triterpenoids with structural differences: Limonoid (D-Ich), Pentacyclic-oleane (OA), pentacyclic-aglycone (18GRA), and saponin (AMO) against Leishmania parasites and infected macrophages. Besides, we evaluated the ultrastructural alterations and physiological properties, by different techniques like transmisión electron microscopy and Flow cytometry. Our results showed *Leishmania (V.) panamensis* infected macrophages increased their size, the presence of vacuoles, and the emergence of pseudopods-like structure, related to activation and early apoptosis death. In the intracellular parasite, we observe intact organelles like a nucleus, membrane, mitochondrion, kinetoplast, axoneme, and the flagellar pocket. On another side, in macrophages exposed to triterpenoids, we identify the presence of membrane protuberances and an increase in the vacuoles system (double

and digestive membranes). While in the intracellular amastigotes, we identify the swelling of the mitochondrial and kinetoplast membrane, as well as the presence of multiple lipid vacuoles and double organelles (dysfunctional cytokinesis). The limonoid causes the appearance of lipid droplets in the host and the pathogen, and the swelling of the kinetoplast membrane, meanwhile OA and 18GRA compounds induce in the macrophages, the presence of double-membrane vacuoles (phagosomes), and electrodence cytoplasms, related with apoptosis processes; while, the effects of the compounds in the parasite, results in the presence of acidocalcisomes-likes forms, doubles organelles and shrink membranes. Finally, AMO saponin affected the mitochondrial membrane potential in infected macrophages and intracellular amastigotes, and the presence of digestive vacuoles in the host and pathogen. These results proved that triterpenes compounds are potential antileishmanial drugs because are capable to induce ultrastructural and physiological changes in the parasite, without causing damage in the macrophage and promoting the killing of *Leishmania*, by the approach to the identification of therapeutic targets.

Keywords LEISHMANIASIS; TREATMENT; *L. (V.) panamensis*; TRITERPENES; ULTRASTRUCTURE

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O34-01: DIAGNOSIS OF VISCERAL LEISHMANIASIS IN AN ELIMINATION SETTING: A VALIDATION STUDY OF THE DIAGNOSTIC ALGORITHM IN INDIA

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Visceral leishmaniasis (VL) is on the verge of elimination on the Indian subcontinent. Nonetheless, the current low VL-incidence setting brings along new challenges, one of which is the validity of the diagnostic algorithm, based on a combination of suggestive clinical symptoms in combination with a positive rK39 Rapid Diagnostic Test (RDT). With this study, we aimed to assess the positive predictive value of the diagnostic algorithm in the current low-endemic setting in India, by re-assessing the newly diagnosed VL patients with qPCR on venous blood as the reference test. In addition, we evaluated the specificity of the rK39 RDT, by testing apparently healthy individuals (without clinical VL) with the rK39 RDT. Participants were recruited in Bihar and Uttar Pradesh, India. VL patients diagnosed based on the diagnostic algorithm were recruited through six Primary Health Care Centers (PHCs); non-VL cases were identified through a door-to-door survey in currently endemic, previously endemic, and non-endemic clusters, and tested with rK39 RDT, as well as, if positive, with qPCR on peripheral blood. We found that 95% (70/74; 95% CI 87-99%) of incident VL cases diagnosed at the PHC level using the current diagnostic algorithm were confirmed by qPCR. Among 15 424 apparently healthy

(without clinical VL) individuals, 39 were rK39 RDT positive, reflecting a specificity of the test of 99.7% (95% CI 99.7 – 99.8%). The current diagnostic algorithm combining suggestive clinical features with a positive rK39 RDT still seems valid in the current low endemic setting in India.

Keywords VISCERAL LEISHMANIASIS; DIAGNOSTIC ALGORITHM; rK39 RAPID DIAGNOSTIC TEST: qPCR

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O34-03: VERY LONG-TERM FOLLOW-UP OF CUTANEOUS LEISHMANIASIS SCARS: IMPACT OF SPECIES, OF RELAPSE AND OF TIME AFTER RECOVERY

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The evolution of cutaneous leishmaniasis (CL) is far different across continents, depending on the parasite species. The decision to treat is based on the need to accelerate the cure and to reduce scarring, and on the risk of dissemination or later progression. Atrophic scars are caused by skin inflammation and result from collagen damage, dermal atrophy, erythema, and fibrosis. CL scars are usually considered permanent, yet they may be either well tolerated, or associated to persistent and disfiguring lesions. Limited data are available in the literature about the effect of various factors such as the *Leishmania* species, the time to heal and the time since the scar healed. Here, we analyze the evolution of the scars according to the *Leishmania* species, the duration of evolution, the observation of relapse or treatment failure. All patients who were diagnosed and managed for CL in Rennes Teaching Hospital (France) during the past 20 years (from 2000 to 2020), were contacted, by phone or mail. Factors associated with the outcome of the scar (invisible, minimal, or persistent/disfiguring) were analyzed using Chi-square test (qualitative data) or two-way ANOVA or Fischer exact test (quantitative data). Rennes Teaching Hospital is located in a non-endemic area, and various species from different continents were identified among 135 patients with CL over the study period: *Leishmania guyanensis*(37%), *Leishmania major* (20%), *Leishmania braziliensis* (10%), *Leishmania infantum* (3%), *Leishmania tropica* (3%), *Leishmania mexicana* (3%), and *Leishmania amazonensis* and *Leishmania naiffi* (both 1%). Patients were treated with IV/IM Pentamidine (3-4 mg/kg x3) in case of

New World CL or intra-lesional glucantime in case of Old World CL (4x1mL per lesion). We could reach out 54 patients 1-5 years (33%), 5-10 years (33%), and 10-20 years (33%) after healing, for interview and photo collection. A total of 35/54 cases (64%) healed without persistent scars after a first course of treatment, and 36% needed further courses because of early failure (mainly with *L. major*) or late relapse (mainly with *L. guyanensis*). Thirty-one % of the lesions were located on the face. The proportion of persistent/disfiguring scars was statistically higher in case of failure/relapse (47% versus 28%, $p<0.05$). *L. guyanensis, L. braziliensis* and *L. major* were statistically associated to failure/relapse and to persistent/disfiguring scars compared to others ($p=0.02$ and $p=0.03$, respectively). For cases due to *L. infantum*, *L. tropica*, and *L. mexicana*, all scars were considered as invisible or minimal. By contrast, the post-healing lapse of time has little impact on the aspect of the long-term scar. It is usually considered that CL induces persistent scars with a substantial social and psychological burden. However, most of the studies on the evolution of CL scars are monocentric and monospecies and with a limited follow-up. Here we show that for 2/3 of patients followed until 20 years after the initial lesion, scars are invisible or with minimal impact. Significant prognosis factors associated to persistent/disfiguring scars were the species (*L. guyanensis, L. braziliensis* and *L. major*) and the need for consecutive courses of treatment due to failure/relapse.

Keywords *Leishmania guyanensis, Leishmania mexicana*; *Leishmania infantum; Leishmania tropica*

O34-04: DIAGNOSTIC PERFORMANCE OF Q5-BASED RAPID DIAGNOSTIC TEST FOR HUMAN VISCERAL LEISHMANIASIS IN ETHIOPIA

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Serological tests have increasingly taken an important place in the diagnosis of visceral leishmaniasis (VL). The ideal test should combine good sensitivity and specificity across all continents and in all patient groups. However, current tests do not achieve this and most perform poorly in HIV coinfected patients. Tests based on recombinant antigens are considered one of the most promising ways for serological diagnosis for VL, as they present high sensitivity and specificity, and – when in rapid diagnostic test format – they are easy-to-use. Previously we engineered a chimeric protein (Q5) and evaluated its VL diagnostic performance in humans and canines through an ELISA assay (Q5-ELISA). In a *Leishmania infantum* endemic region of Brazil, it showed a sensitivity of 82% and a specificity of 99%. The present study aimed to evaluate an alternative rapid diagnostic test based format (Q5-RDT) in a *L. donovani* endemic region in Ethiopia. To determine sensitivity, a panel of 76 stored serum samples from patients with VL (47 coinfected with HIV and 29 without HIV) was selected. VL diagnosis of these samples was confirmed by microscopy or PCR on tissue aspirates. Specificity was evaluated using samples from healthy endemic controls (n = 10), non-endemic controls (n = 10), and patients with confirmed malaria infection (n = 10). Samples were also tested using rK39-RDTs, with overall sensitivity

varying between 89.0% (Kalazar Detect) and 91.2% (IT Leish) and specificity varying between 92.5 (Kalazar Detect) and 100% (IT Leish). The overall sensitivity of the RDT-Q5 test reached 76.3% (95% CI 65.6-84.5%) (74.5% for VL-HIV and 79.3% for VL only) and specificity was 100% (95% CI 88.6-100%). In conclusion, the RDT-Q5, initially developed for use in Brazil (*L. infantum*), maintained a comparable performance for diagnosis of VL due to *L. donovani* in Ethiopia – including in VL-HIV co-infected patients, though with a decrease in sensitivity in the latter. In addition, the performance of the RDT-Q5 was lower compared to the conventional rK39 rapid tests, often recommended in diagnostic guidelines. Because the specificity was satisfactory, a larger sample set is recommended for validation. Finally, modifications of the chimeric protein are needed aiming to increase sensitivity.

Keywords POINT-OF-CARE; VISCERAL LEISHMANIASIS; DIAGNOSIS; CHIMERIC PROTEIN

O34-05: SEROLOGICAL DIAGNOSIS OF VISCERAL LEISHMANIASIS: GOOD ALTERNATIVE WITH THE RECOMBINANT PROTEIN KR95

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Leishmaniasis is an important vector-borne disease with worldwide distribution, and the clinically most severe form is visceral, affecting internal organs and leading to death in untreated cases. In Brazil, Visceral Leishmaniasis (VL) is caused by the *Leishmania infantum* and is distributed

in all country regions. In 2020, Brazil reported 1,933 VL cases, representing more than 97% of cases in the Americas, and the lethality for the same period was 9.5%. It is a potentially fatal disease, and an accurate diagnosis is essential to provide the appropriate treatment. Serological methods such as immunochromatographic tests are good for VL diagnosis, but the performance may vary depending on the host factors and the geographic region where the infection was acquired. Thus, evaluation of diagnostic alternatives is essential. The objective of this study was to evaluate ELISA performance with not much-studied recombinant antigens, K18 and KR95 (IDRI, USA). For comparison purposes, we used two already known recombinant proteins, rK28 and rK39 (IDRI, USA). After standardization and verification of the best test condition for each antigen, sera from parasitologically confirmed symptomatic VL patients (N=90) and healthy endemic controls (N = 90) were submitted to ELISA. The sensitivity of the rK18-ELISA and rKR95-ELISA was, respectively, 83.3% and 95.6%, and specificity was 93.3% and 97.8%. For validation of ELISA with the recombinant antigens, samples from five states in Brazil (Rio Grande do Norte and Sergipe (Northeast region), Rio de Janeiro and Sao Paulo (Southeast region), and Mato Grosso do Sul (Midwest region) were included: 122 from VL patients and 83 from healthy controls. Comparing the results obtained by rK18-ELISA, rKR95-ELISA, rK28-ELISA, and rK39-ELISA in the VL patients 122 samples, the sensitivity of the rKR95-ELISA (95.9%; 95% CI: 90.5%-98.5%) was high and compared with rK28-ELISA (96.7%; 95% CI: 91.6%-99.0%) and rK39-ELISA (97.5%; 95% CI: 92.7%-99.5%) no significant differences were obtained but, when compared with rK18-ELISA, a lower sensitivity ($p < 0.001$; Cochran's Q test) was obtained. In the 83 health controls samples, the biggest specificity was obtained by rKR95-ELISA (96.4%; 95% CI: 89.5%-99.2%) and lowest by rK18-ELISA (62.7%; 95% CI: 51.9%-72.3%) ($p < 0.001$; Cochran's Q test) when compared with rK28-ELISA (95.2%; 95% CI: 87.9%-98.5%) and rK39-ELISA (95.2%; 95% CI: 87.9%-98.5%). No difference across localities (chi-square test, $p>0.05$) was observed in sensitivity and specificity in the rKR95-ELISA test. Cross-reactivity assessment was performed with sera of patients diagnosed with inflammatory disorders and other infectious diseases: autoimmune disease (N=10); Chagas disease (N=47); cutaneous leishmaniasis (N=28); malaria (N=12); mucosal leishmaniasis (N=14); paracoccidioidomycosis (N=27);

syphilis (N=20); toxoplasmosis (N=20); active pulmonary tuberculosis (N=12). The cross-reactivity with other diseases was 34.2% by rK18-ELISA and 3.1% by rKR95-ELISA. Unlike the rK18-ELISA, the recombinant KR95 antigen in the ELISA test showed excellent diagnostic accuracy and can be considered a good alternative for VL diagnosis.

Keywords VISCERAL LEISHMANIASIS; ELISA; RECOMBINANT ANTIGENS; DIAGNOSIS

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O34-06: COST EFFECTIVENESS ANALYSIS OF AMBISOME AND MILTEFOSINE COMBINATION THERAPY VS AMBISOME MONOTHERAPY FOR THE TREATMENT OF VL-HIV CO-INFECTION IN INDIA

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Visceral leishmaniasis (VL) in patients with human-immunodeficiency-virus (HIV) presents an increasingly important public health issue in areas where both infections are endemic. A recent randomized clinical trial from the Indian setting has been published suggesting that for this patient group, a combination of liposomal amphotericin B (AmBisome) and miltefosine (MF) is a safe and effective alternative to the current recommended AmBisome monotherapy. This study estimates the cost-effectiveness of both treatment regimens in the Indian context. Two alternative treatment methods were considered in the treatment of patients co-infected with VL-HIV. In one treatment method, patients were administered 5 mg/kg of AmBisome on eight days throughout a 24-day period, resulting in a total dose of 40 mg/kg.). The second therapy consisted of a combination of 5 mg/kg of AmBisome administered on six days throughout an 11-day period for a total dose of 30 mg/kg along with 50 mg of MF administered orally twice a day for 14 days for a total dose of 1.4g. This combination is the current WHO recommended standard of care in India and East for VL-HIV. A decision tree model was developed using TreeAge Pro Healthcare, to compare the cost-effectiveness of the two treatment strategies. The probabilities for variables in the decision tree were obtained primarily from a published randomized study in Bihar, India that looked at the safety and efficacy of the two treatment regimens for VL in patients coinfected with

HIV. Probabilities were also obtained from expert opinion and published literature. Considering the importance of Tuberculosis (TB) co-infection on outcomes, patients were first classified as being positive or negative for TB. The patient is then classified as either adhering to the treatment method or not adhering. The effectiveness of a treatment method was expressed in terms of treatment failure (death and relapses averted). In addition to the baseline analysis, a Monte Carlo probabilistic sensitivity analysis was performed with 10,000 iterations. Variables in the sensitivity analysis were assigned distributions to account for uncertainty in the model and were randomly sampled at each iteration. Cost variables as well as variables concerning the compliance to treatments, effectiveness of treatments, and prevalence of TB were varied in the sensitivity analysis. Direct medical costs, such as the cost of drugs and the cost of inpatient beds per day were varied based on the data from MSF and WHO sources. Indirect costs were also varied to account for variances in our sources. The costs and effectiveness of both treatment methods were compared in the baseline analysis. The combination therapy had a lower cost and higher effectiveness than the monotherapy, thus being the dominant treatment method. The combination therapy had an effectiveness of 94% whereas the monotherapy had an effectiveness of 84%. The combination therapy also proved to be less costly than the monotherapy. The sensitivity analysis showed that the model was robust, with the iterations showing dominance of combination therapy. AmBisome/miltefosine combination therapy appears to be more cost effective than AmBisome monotherapy for the treatment of VL-HIV in the Indian setting.

Keywords VISCERAL LEISHMANIASIS; HIV; TREATMENT; COST-EFFECTIVENESS

O41-01: EFFECTIVENESS OF ANTILEISHMANIAL TREATMENTS IN THREE ENDEMIC AREAS OF COLOMBIA

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Control of cutaneous leishmaniasis (CL) in the Americas largely relies on passive case detection and ambulatory treatment. Information on clinical response under the standard of care is scarce. Logistical constraints of endemic areas have contributed to the virtual absence of information on routine effectiveness of antileishmanial drugs, as opposed to efficacy. This project sought to estimate the effectiveness of the standard treatment for CL in three municipalities of Colombia (Pueblo Rico in Risaralda, Rovira in Tolima, and Tumaco in Nariño), supported with mHealth tools managed by members of the community. Confirmed CL patients who received standard antileishmanial treatment were eligible for this observational study. Trained community health leaders supported patient follow-up in rural areas of three municipalities using a mobile application (Guaral+ST app) or paper forms. Therapeutic response was assessed by physicians, either remotely using photographs taken with the app, or in face-to-face visits at days 90 and/or 180 post treatment. We present preliminary results of 202 enrolled participants. Among them, 56 received miltefosine, 85 meglumine antimoniate (MA) and 61 pentamidine. In terms of ethnicity, participants were mostly Afro-Colombian (34.5%), followed by *mestizo* (32.9%) and indigenous (30.8%). The median age was 19 years (IQR: 9-28). Disease presentation was mild with the median number of lesions being 1 (IQR: 1-2) and short disease duration (median 1.5 months; IQR: 1-3). Therapeutic failure occurred in 13.3% (6/45; 95% CI: 5.9-27.3%) for miltefosine; 28.1% (18/64; 95% CI: 18.2-40.6) for MA and 16.7% (9/54; 95% CI: 8.7-29.5) for pentamidine. The community-based approach yielded effectiveness

information on 81% of patients. Analysis of factors associated with loss to follow-up, and more detailed analysis of effectiveness, are in progress. Results of this project provide evidence of treatment effectiveness of antileishmanials in real-life conditions. The point estimate of the proportion of treatment failure with meglumine antimoniate was higher than pentamidine or miltefosine but confidence intervals overlap. Our findings support the feasibility of community-based assessment of effectiveness of treatment through task-shifting to community members using mHealth tools.

Keywords CUTANEOUS LEISHMANIASIS; EFFECTIVENESS; ANTILEISHMANIAL; TREATMENT; COLOMBIA

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O41-02: ETHNOPHARMACEUTICALS FOR THE TREATMENT OF CUTANEOUS LEISHMANIASIS OF THE OLD WORLD

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Toxicity, expense, and accessibility limit treatment success in Old World Leishmania infections in the Middle East, Mediterranean basin, Arabian Peninsula, Africa as well as the Indian Subcontinent. Better drugs are urgently needed, however, drug discovery is hampered by limited funding given geographic restriction of highly endemic OWCL to LMICs. Plant-based compounds with potential anti-leishmanial effects found in and around local endemic communities present an opportunity to overcome the aforementioned therapeutic challenges, and many such interventions are supported by anecdotal evidence of efficacy. We aim to synthesize existing evidence around available ethnopharmaceuticals to promote drug discovery for the prevention and treatment of OWCL. Four electronic databases were searched for studies reporting the efficacy, safety, time of lesion resolution or tolerability of ethnopharmaceuticals. Studies were systematically screened, followed by data extraction. Trial quality was assessed using the GRADE approach. We conducted a narrative synthesis of studies reporting efficacy, size of lesion, time of lesion resolution and adverse events. Data were summarized using qualitative and quantitative measures. 13 studies were included evaluating a number of topical applications of ethnopharmaceuticals including: Buca (Mat lippie), Cassia

fistula, Z-HE, Juniperus excelsa, honey, Achilles millefolium, ozonated olive oil, Sambucus ebulus, garlic, Azadrachta indica, Acacia nilotica, Physalis minima and Morinda citrifolia. Eight (62%) studies were RCTs, 3 (23%) studies were cohorts and 2 (16%) studies were from patents. C. fistula gel was the most studied extract, evaluated in addition to Glucantime therapy, where topical gel resulted in complete cure [RR = 1.62 (1.17-2.24)]. Synthesizing the current evidence surrounding ethnopharmaceuticals for the treatment of OWCL may contribute to drug discovery pipelines and potentially lead to novel therapeutics in a field that has not seen any new drug development for over half a century.

Keywords CUTANEOUS LEISHMANIASIS; OLD WORLD; ETHNOPHARMACEUTICALS

4.3 DRUG DISCOVERY & DEVELOPMENT

O2-02: THE PAST BUILDS THE FUTURE: TAMOXIFEN/CLEMASTINE CHIMERA AS A POTENTIAL ANTILEISHMANIAL TREATMENT

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The range of drugs available to treat leishmaniasis are far from ideal: they induce lethal side effects, require special infrastructure due to parenteral administration and some of them have been showing a decrease on responsiveness to treatment. Collectively, these shortcomings make the discovery of new alternative treatments an urgent matter. The search for new drugs is expensive, laborious, time-consuming and risky. Therefore, using existing approved drugs is an attractive strategy to accelerate drug discovery. In this context, tamoxifen, a selective oestrogen receptor modulator (SERM) and known anti-breast cancer drug, has been identified as a potent anti-leishmanial, displaying significant activity against both *in vitro* and *in vivo* infection models. Similarly, clemastine fumarate, an over-the-counter first-generation antihistamine drug, has submicromolar activity against intramacrophage amastigotes of *Leishmania amazonensis* as well as equivalent activity to glucantime in a mouse model infection. Interestingly, both molecules display similar chemical features and have also been proposed to target the same enzyme: the inositol phosphorylceramide synthase (IPCS), an essential enzyme to the parasite which is part of the

sphingolipids biosynthetic pathway. However, previous studies have shown that clemastine and tamoxifen have multiple intracellular targets. These could lead to toxicity and lack of selectivity, and are yet to be explored. To investigate this in greater detail – and develop more effective selective compounds –, we have built a library of tamoxifen/clemastine hybrids based on the common chemical features shared by these molecules. Following initial screening against *L. major* and *L. amazonensis* promastigotes, as well as cytotoxicity assays using HepG2 cells, several hybrids have shown submicromolar activity and no toxicity against human cells. This showed an improvement from parental molecules, which are toxic against HepG2 cells. The most active compounds (EC_{50} < 2 µM against both species of promastigotes together with SI > 10 *versus* Hep G2) are currently being tested against intracellular amastigotes. This presentation will describe these studies together with the ongoing experiments designed to explore the mode of action and molecular target(s) of these chimeric compounds.

Keywords SELECTIVE OESTROGEN RECEPTOR MODULATOR; HEP G2; INOSITOL PHOSPHORYLCERAMIDE SYNTHASE

O2-03: NATURAL PRODUCTS AGAINST *Leishmania (L.) infantum*: THE STUDY OF LICARIN A SEMI-SYNTHETIC DERIVATIVES

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Leishmaniasis is a protozoan parasitic disease caused by *Leishmania* spp. and has been included among the six most important neglected tropical diseases by WHO. The treatment of relies on few chemotherapeutic agents, with mild to severe side-effects. In this context, natural metabolites from plants represent a promising inspiration for the design of new drug candidates. Three semi-synthetic derivatives of the natural neolignan licarin A were prepared: O-acetyl (1a), O-allyl (1b), and 5-allyl (1c). Using an *ex vivo* assay, compounds 1a, 1b and 1c showed activity against the intracellular amastigotes of *Leishmania (L.) infantum*, with IC_{50} values of 9, 13 and 10 µM, respectively. Despite no induction of hemolytic activity, only compound 1b resulted in mammalian cytotoxicity, resulting in a CC_{50} value of 64 µM. The most potent compounds (1a and 1c) presented selectivity indexes >18. Based in these results, 1c was selected to a mechanism of action (MoA) study using MALDI-TOF/MS and fluorescent/luminescent techniques as flow cytometry. After 1-2h incubation with promastigotes, 1c induced increased levels of the cytosolic calcium, with alkalinization of the acidocalcisomes. Hyperpolarization of mitochondrial membrane potential, followed by decreased levels of ATP were also observed, without alterations of reactive oxygen species levels. The permeability of the plasma membrane was not affected, and no DNA fragmentation was observed, but the cellular proliferation was highly compromised. Mass spectral alterations of *Leishmania* proteins were observed, but differently from those obtained with miltefosine, suggesting a different MoA. This chemically modified neolignan (1c) induced lethal alterations of the bioenergetic and protein

metabolism of *Leishmania.* In conclusion, natural product-based drug discovery has been considered a promising tool for the selection of new hits against Leishmania and licarin A derivatives may be considered for future optimization studies.

Keywords *Leishmania (L.) infantum;* NATURAL PRODUCTS, LICARIN A, MECHANISM OF ACTION

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O2-04: COMBINATION THERAPY FOR CHAGAS DISEASE – PYRROLOPYRIMIDINE SERIES

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Chagas disease or American trypanosomiasis is a potentially life-threatening disease caused by *Trypanosome cruzi*. It is estimated that 6-7 million people are infected with *T. cruzi*, mostly in Latin American countries where the disease is endemic. *T. cruzi* infection is curable if treatment is initiated soon after infection, during its acute phase, benznidazole and nifurtimox being the only available treatments. However, treatments are long (up to 2 months), efficacy diminishes the longer a person has been infected and nearly all of treated adult patients suffer from adverse reactions, some of which can lead to interruption of the treatment. Additionally, neither benznidazole nor nifurtimox should be taken by pregnant women nor people with kidney or liver failure. Hence, oral, efficacious, shorter and safer treatments for Chagas disease are urgently needed. Combination therapy is attractive since it can Improve treatment

efficacy Reduce dose and/or duration resulting in fewer adverse effects for current standard of care is a validated approach for anti-infectives compounds. In our search for novel and shorter treatments for Chagas disease, and in collaboration with university of Dundee and Drugs for Neglected Diseases initiative (DNDi), we optimized a chemical series that originated from our phenotypic *in vitro* screening of 1.8M molecules [Peña, I *et al. Sci Rep* 5, 8771 (2015) that led to the identification of TCMDC-143610. This pyrrolopyrimidine series is active across different kinetoplastid parasites, such as *Leishmania spp* and *T. cruzi* with a novel mechanism of action. Initial hit was optimized improvinag its potency and metabolic stability profile enabling progression to a bioluminescent Chagas *in vivo* model [Lewis, M. D *et al*. *Cellular microbiology* vol. 16,9 (2014): 1285-300]. Compounds from this chemical series were able to show no relapse in mice after just 5 days of treatment in combination with a suboptimal dose of benznidazole opening the door to short treatments for Chagas disease.
"All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals."

Keywords CHAGAS; COMBINATION; *Trypanosoma cruzi;* PYRROLOPYRIMIDINE.

O2-05: IDENTIFYING AND VALIDATING NEW DRUG TARGETS FOR THE TREATMENT OF LEISHMANIASIS

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With a clear link with poverty and limited investment in new methods for treatment, leishmaniasis is one of the most neglected diseases and represents a major unmet global health challenge. There are currently no prophylactic vaccines and chemotherapies in current use have restricted efficacy, difficult modes of administration, are expensive and are not widely accessible. In addition, drug toxicity and emerging resistance are also major concerns. Consequently, there is a major need for new tractable drug targets and associated drugs. Whilst numerous compounds, of both natural and synthetic origin, with antileishmanial activity are reported each year relatively few are linked with a molecular target. Many of these compounds contain intrinsic reactive functionalities and we have exploited these to develop chemical probes to label and identify new drug targets and putative modes of actions. In this presentation we will describe selected examples of this approach describing the synthesis of the probes and their subsequent application to target discovery in *Leishmania* spp. In a phenotypic approach, we have used a natural chalcone, with antileishmanial properties, as a starting point to develop and apply probes to identify and validate the enzyme tryparedoxin peroxidase (cTXNPx) as a putative drug target. In a second and complementary, target-based strategy, we have previously identified the essential kinetoplastid sphingolipid synthase (SLS) as an attractive pharmaceutical target due to the divergence of function compared with the mammalian orthologue. We have developed screening assays to identify potential inhibitors with good levels of activity against multiple *Leishmania* species including *L. major, L. amazonesis and L. donovani*. Each compound has been elaborated to provide probes to explore the mode of action and / or the molecular target in the parasite. Details of these studies

together with further progression of these hits towards drug leads will be presented.

Keywords *Leishmania*; DRUG-DISCOVERY; TARGET-IDENTIFICATION; CHEMICAL PROBE

O2-06: CROMALEISH®, A NEW PROTOTIPE OF TOPICAL FORMULATION TO TREAT CUTANEOUS LEISHMANIASIS

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Cutaneous leishmaniasis (CL) is an endemic infection in several countries of the world. Due to variable response to therapy and frequency of relapses a more effective, safe, and inexpensive treatment is needed. Previously it was reported that the hederagenin glucoside saponins (SS) and chromane hydrazone (TC2) combined in a 1:1 ratio has high potential in antileishmanial therapy since both compounds alter the survival of *Leishmania* and the ability to infect adjacent macrophage. In this work, we developed an ointment formulation containing 2% TC2 and 2% SS (w/w) and determined the skin permeation and the absorption but also the acute dermal toxicity by in vitro and in vivo assays. Last, the effectiveness and safety of the topical therapy to treat non-complicated CL was evaluated in an observational study in human and canine patients from endemic areas of Colombia. Both TC2 and SS diffused through pig ear skin and traces of TC2 but not SS were detected in the *stratum corneum* of mice at 6 – 24 hours. Neither TC2 nor SS were detected in plasma. The acute dermal toxicity was negative. Treatment with 2% TC2 – 2% SS ointment produced a complete long-term clinical cure in 10 patients (4 women and 6 men) and 56 dogs (24 females and 32 males) without adverse effects. All human and canine patients have remained disease-free for the last 24 months. In conclusion, these results support the use of topical therapy as a safer and new first-line local treatment of CL that could be further validated by controlled clinical trials.

Keywords CANINE CUTANEOUS LEISHMANIASIS; SAPONINS; CHROMAN HYDRAZONE; TOPICAL TREATMENT

Financing Universidad de Antioquia (AI-51890) and Minciencias (CT-449-2021)

O9-01: ANTILEISHMANIAL ACTIVITY IN VITRO OF ARNICA TINCTURE AND ITS THERAPEUTIC EFFECTIVENESS IN EXPERIMENTAL CUTANEOUS LEISHMANIASIS CAUSED BY *Leishmania braziliensis* AND *L. tropica*

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Arnica tincture (AT), an ethanolic extract prepared from the flowerheads of *Arnica montana* L., Asteraceae, is a traditionally used herbal medicine for the topical treatment of injuries and inflammations. Previous studies have shown antileishmanial activity of AT and isolated Arnica sesquiterpene lactones (STLs) in *Leishmania braziliensis* and *L. infantum*, respectively. Moreover, AT showed comparable or even better curative effects than the standard drug glucantime, in experimental cutaneous leishmaniasis (CL) in golden hamsters infected with *L. braziliensis*. CL treatment has long relied on the use of pentavalent antimonials. Nonetheless, the response of *L. braziliensis* and *L. tropica* to pentavalent antimonials is increasingly poorer, and therefore new and more potent therapeutic alternatives are needed. In this work, we studied the in vitro cytotoxicity and antileishmanial activity of AT and STLs against both *L. braziliensis* and *L. tropica* using MTT assay and flow cytometry, respectively. The in vivo therapeutic effect of the arnica tincture was studied in hamsters experimentally infected with *L. braziliensis* and *L. tropica*. The STLs and the AT possess a very high activity against both Leishmania species with median effective concentrations (EC_{50}) ranging from 1.9 to 5.9 □g/mL. The AT was not cytotoxic for human tissue macrophages, skin fibroblasts, and hepatic cells. It did not show any irritant or corrosive potential for the skin. The therapeutic response of hamsters infected with *L. braziliensis* or *L. tropica* to the treatment with AT in

hamsters was 87.5% (at a dose of 19.2 □g/2X day/60 days) and 72.7% and 67% at doses of 19.2 □g/day/60 days and 38.4 □g/2x day/60 days, respectively. In turn, the effectiveness of treatment with glucantime administered intralesionally at a dose of 200 mg/every three days for 30 days was 50% to *L. braziliensis* or *L. tropica* infection. These results are promising and encourage the continuation of clinical trials with AT in CL patients to turn AT into a leishmanicidal drug.

Keywords *Arnica montana*; NATURAL PRODUCTS; PHYTOMEDICINE; SESQUITERPENE LACTONES; *Mesocricetus auratus*; GOLDEN HAMSTER

Financing The Wilhelm Doerenkamp-Foundation (Natvantage Research Grant 2018)

O9-02: PREDICTION OF EXPERIMENTAL MODELS FOR THERAPEUTIC STUDY WITH CHALCONES BASED ON METABOLISM

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Chalcones have been extensively demonstrated in silico, in vitro and rodent animal models as a novel class of antileishmanial drugs. We have recently synthesized an active nitro chalcone analogue NAT22 with strong binding and inhibitory activity of tryparedoxin peroxidase (cTXNPx), an important parasite redox enzyme. Surprisingly, when used in mice to treat cutaneous leishmaniasis, NAT22 was more effective by oral than intralesional route. Liver metabolism differs among species, so here we proposed to compare *in silico* and *in vitro* NAT22 metabolites generated by mouse, dog and human liver microsomes in order to recognize mouse as relevant animal model in oral chalcone studies. Also, parasite NAT22 metabolism was also studied in terms of nitroreductase (NTR) enzyme using NTR++ *Leishmania infantum*. *In silico* prediction of human liver metabolites using ADMET Predictor software showed NAT22 to be a substrate for CYP 1A2, 2A6, 3A4 e 2C8 liver enzyme. The predicted human metabolites were one hydroxylated (novel) and two demethylated (one novel) compounds. *In vitro*, NAT22 was incubated with mouse, dog and human liver microsomes together with NADPH for 90 min / 37°C. Several metabolites were identified by LC-MS/MS and/or NMR. Although no relevant qualitative interspecies differences were found, human microsomes metabolized more NAT22 than dog and mouse microsomes. In cellulo, NTR++ promastigotes were more susceptible to NAT22 than WT parasites, suggesting a role for parasite NTR in NAT22 detoxication. One of three predicted metabolites were synthesized but shown to be as active as NAT22. In sum, these studies suggest that humans

may respond better to oral treatment with chalcone NAT22 than mice, and that Leishmania parasites may partially inactivate native NAT22 through their NTR.

Keywords DRUG LIVER METABOLISM; LIVER MICROSOMES; LC-MS/MS; *Leishmania*; NITRO CHALCONE

O9-04: EVALUATION OF TWO FDA-APPROVED ANTISEPTICS USED IN WOUND CARE AND ORAL HYGIENE FOR EFFICACY IN THE TREATMENT OF LEISHMANIASIS

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Leishmaniasis affects poor communities worldwide, with about 1 million new cases annually and more than 20 species of the parasite affecting humans. In infected hosts, the parasites exist in 2 forms-promastigotes in blood and amastigotes in tissue macrophages. The disease manifests in 3 clinical forms: Cutaneous (most common), Mucocutaneous and Visceral (most serious). Available therapies are very toxic, expensive, selective for specific species of the parasites, and often complex to administer. Hence, effective treatment requires specific identification of parasite specie (difficult in resource-limited areas), long-term administration by adequately skilled personnel and severe side effects, often leading to patient non-compliance. New drugs are desperately needed, and drug repurposing could offer quick intervention. We identified two compounds that are commonly used in wound care and oral hygiene that have impressive anti-leishmanial activity in vitro. The anti-leishmanial effects of the compounds were initially assayed on promastigote forms of the parasites. Parasite growth and/or inhibition was monitored using Alamar Blue stain in a fluorescent spectrophotometer. Results of promastigote assays show that the two compounds have better anti-leishmanial activity than some currently available drugs which are often toxic and expensive, with an IC50 of 0.5-2.5□M (1.5-2.5□g/ml). These compounds have shown efficacy against 8 different species of Leishmania tested so far, hence could offer broad-spectrum therapeutics for Leishmaniasis. The safety of these compounds has also been well established, being currently approved and used in wound care and surgical procedures. Experiments are underway to test the

compounds against amastigotes and in mouse models of Leishmaniasis, and to elucidate their mechanism(s) of action. The compounds will also be assayed for synergistic effects with other antimicrobials for enhanced efficacy and drug delivery. This study will deliver two prospective drug-repurposing candidates that are cheap, safe, easy to administer and have broad-spectrum anti-leishmanial activity for the effective treatment of various forms of Leishmaniasis, thereby reducing the disease burden.

Keywords DRUG REPURPOSING; BROAD-SPECTRUM; ANTI-LEISHMANIA, PROMASTIGOTES; FDA-APPROVED

O9-06: A PHASE I, SINGLE ORAL ASCENDING DOSE STUDY IN HEALTHY SUBJECTS OF THE BENZOXABOROLE DERIVATIVE DNDI-6148, A NOVEL DRUG CANDIDATE FOR LEISHMANIASIS

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Leishmaniasis, a parasitic disease caused by Leishmania species and transmitted by female sandflies, mainly affects populations with poor access to good health care systems. It is endemic in three main geographic areas (Eastern Africa, Latin America, and South Asia). Visceral leishmaniasis (VL) is the most severe form of the disease. Existing treatments are based on sodium stibogluconate, meglumine antimoniate, paromomycin, liposomal amphotericin B and miltefosine. However, all these marketed drugs have significant limitations and varying levels of efficacy when used as monotherapies or in combination. Together with industrial and academic partners, DNDi is developing an unprecedented portfolio of drug candidates, aiming for novel, orally active, simpler and safer therapies that will transform leishmaniasis treatment in all endemic regions. One of these candidates, DNDI-6148, is a novel benzoxaborole derivative (Mowbray et al., J. Med. Chem. 2021, 64 (21): 16159-16176) that acts principally through the inhibition of Leishmania cleavage and polyadenylation specificity factor (CPSF3) endonuclease. DNDI-6148 is being investigated for the oral treatment of visceral leishmaniasis in human. A first-in-human phase 1 clinical study investigated the safety, tolerability and pharmacokinetics of single ascending doses of DNDI-6148 in healthy male subjects. The trial was conducted in France in compliance with ICH guidelines and relevant EU

directives. A single oral dose of DNDI-6148 (10 to 380 mg) or placebo was administered to 8 subjects (randomized 6:2) in 8 cohorts, for a total of 64 subjects. DNDI-6148 and placebo, presented as powders for oral suspension, were suspended extemporaneously in Orasweet™ prior to administration. Primary endpoints included safety and tolerability variables (adverse events, physical and neurological findings, vital signs, electrocardiograms and clinical laboratory parameters) and pharmacokinetics variables calculated from plasma concentrations over time. Overall, DNDI-6148 was found to be well tolerated and safe with 14 (21.9%) subjects reporting adverse events, all mild or moderate in severity and mainly unrelated to the drug. Absorption time increased from 3 to 9 hours with increasing doses and was followed by a multiphasic decrease in plasma concentrations. A dose-proportional increase in exposure (AUC and Cmax) and a terminal half-life of approximately 23 hours were observed. The apparent volume of distribution of DNDI-6148 was between 50 and 100 L. Another study in healthy subjects is now under preparation to assess safety and pharmacokinetics of DNDI-6148 after multiple doses. If proven safe and with appropriate exposure, this compound is expected to progress to clinical development in VL patients, possibly as monotherapy or in combination with other new chemical entities, and potentially in other indications such as Chagas disease or cutaneous leishmaniasis.

Keywords LEISHMANIASIS; NEW CHEMICAL ENTITY; SAFETY; FIRST-IN-HUMAN; PHARMACOKINETICS
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O15-01: SEMI-MECHANISTIC PK MODELLING OF DRUG-MACROPHAGE INTERACTIONS AFFECTING THE DISPOSITION OF LIPOSOMAL AMPHOTERICIN B IN PLASMA AND SKIN TISSUE OF POST KALA-AZAR DERMAL LEISHMANIASIS PATIENTS

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The efficacy and safety of shortened liposomal amphotericin B (Ambisome®; LAmB) regimens for post kala-azar dermal leishmaniasis (PKDL) is under investigation on the Indian subcontinent. The pharmacokinetic (PK) properties of LAmB in leishmaniasis patients remain unclear. It is known that the mononuclear phagocyte system (MPS) generally plays a pivotal role in the disposition of liposomal drugs. The influence of PKDL pathophysiology on the plasma and skin target-site pharmacokinetics of LAmB has not been investigated. This study aimed to characterize the PK of LAmB in plasma and skin of PKDL patients and the effects of disease recovery using a population PK modeling approach. The PK data originated from a clinical trial studying short course LAmB monotherapy versus LAmB plus miltefosine combination therapy for PKDL treatment in Bangladesh and India. LAmB was given intravenously twice per week for 2 weeks at a total dose of 20 mg/kg (5 x 4 mg/kg). Total amphotericin B concentrations, including free, tissue-bound, protein-bound and liposomal amphotericin B, in plasma and skin were analyzed. Plasma samples were collected after the first and the last LAmB administration. One

skin biopsy was taken at the end of last LAmB infusion for patients allocated in the monotherapy arm, and at 1 week after last infusion for patients allocated in the combination therapy arm. Population PK analysis was performed using nonlinear mixed effects modelling. PK data from 60 patients were analyzed. Non-linearities in LAmB PK within and between sampling intervals were observed. A model with saturable distribution towards the peripheral compartment, representing drug accumulation in macrophages, best described the data. The maximal drug accumulation in this assumed macrophage compartment (B_{max}) was estimated at 91±7.5 mg of LAmB. A decrease in B_{max} over time was found resulting in a 25% lower B_{max} at end of treatment, reflecting a treatment effect on the activity of macrophages. Median amphotericin B concentration in skin at end of treatment was 7.11 µg/g (range 1-360 µg/g). In total, 96% of the skin observations were above reported in *vitro* IC_{50} against amastigotes *L. donovani* (0.09-0.36 mg/L). A trend of higher C_{max} in plasma after the last infusion with higher exposure in skin was observed. Estimated drug elimination half-life in skin was 43-fold longer compared to plasma (346 hour in skin versus 8 hours in plasma), indicating a much longer residence time of the drug at the target site in skin tissue compared to plasma. PK of LAmB in plasma and skin were elucidated in leishmaniasis patients for the first time. The present model suggested that LAmB follows non-linear PK characteristics of liposome disposition, driven by saturation of macrophage uptake and opsonization. Besides, the model suggested that maximal LAmB accumulation in macrophages decreases during the treatment period. We identified a much longer residence time of LAmB in the skin than in plasma, indicating that drug exposure at the skin target site cannot be simply informed by plasma PK. This highlights the importance of target site PK studies in PKDL patients and other dermal forms of leishmaniasis.

Keywords LIPOSOMAL AMPHOTERICIN B; PKDL; POPULATION PHARMACOKINETICS; SKIN PHARMACOKINETICS

O15-02: CUTANEOUS LEISHMANIASIS TREATMENT AND THERAPEUTIC OUTCOMES IN SPECIAL POPULATIONS: A COLLABORATIVE RETROSPECTIVE STUDY

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Treatment guidance for children and older adult patients affected by cutaneous leishmaniasis (CL) is unclear because they are poorly

represented in clinical trials. RedeLEISH, a Latin American network of investigators in leishmaniasis, conducted a collaborative retrospective study to describe the effectiveness and safety of antileishmanial treatments in children and older adults, treated between 2014 and 2018 in ten CL referral centers in Bolivia, Brazil, Colombia and Peru, with the goal of providing recommendations for improved management of these CL patients. The study protocol was approved by the institutional ethics committee of each participating institution. The study included patients aged ≤10 or ≥60 years at the time of treatment, with a parasitological or clinical and epidemiological diagnosis of uncomplicated CL, and at least one evaluation of therapeutic response at any time after completion of treatment. Data entry was performed in an offline data collection tool in Microsoft Access. Statistical analyses were performed using Stata/SE, version 15 (StataCorp LP, College Station, TX), and statistical analysis plan was agreed prior to analysis in alignment with the protocol. Eligibility was assessed for 2,037 clinical records. The main reason for non-inclusion was lack of data on treatment follow-up and therapeutic response (75% of children -182/242 and 38% of adults -179/468). Data on 1,325 eligible CL patients (736 children and 589 older adults) were analyzed. In both age groups, disease presentation was mild, with a median number of lesions of 1 (IQR: 1-2) and median lesion diameter of less than 3 cm. Data for two or more post-treatment follow-up visits was available for less than 50% of the patients and only 28% of the children. Systemic antimonials were the most common monotherapy regimen used in both age groups (80.2%, 590/736 children and 52.3%, 308/589 older adults). Intralesional antimonials were administered to 14.5% (n=84) of adults and 2.2% (n=16) of children. Other treatments included miltefosine, amphotericin B and pentamidine. Overall cure rates for systemic antimonials were 54.6% (95% CI: 50.46-58.6%) in children and 68.2% (95% CI: 62.6-73.39%) in older adults. In patients aged ≥60 years, an overall cure of 84.5% (95% CI: 74.99-91.5%) was observed for intralesional antimonials. The frequency of adverse events (AE) related to the treatment was 11.9% (86/722) in children versus 38.4% (206/537) in older adults. AEs were mainly of the types previously reported, and mostly of mild intensity. Our findings on the predominance of mild disease presentation and limited effectiveness of systemic antimonials in pediatric and older adult patients support the need for wider implementation of local

therapies and greater availability of alternatives to systemic antimonials. This study also highlights the need to develop strategies to improve the patient's adherence to clinical follow-up across the region, with special attention to the pediatric population. This initiative provides a proof of concept of the benefits of regional data sharing to address clinical questions that are otherwise difficult to achieve at individual study sites.

Keywords CUTANEOUS LEISHMANIASIS; TREATMENT; OUTCOME; CHILDREN; OLDER ADULT

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O15-03: EFFICACY AND SAFETY OF 14 DAYS OF PAROMOMYCIN AND MILTEFOSINE FOR THE TREATMENT OF PATIENTS WITH PRIMARY VISCERAL LEISHMANIASIS IN EASTERN AFRICA

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Visceral leishmaniasis (VL) is a parasitic disease caused by *Leishmania donovani* in Eastern Africa, currently the region with highest burden worldwide. It mainly affects children and is fatal if not treated. Current SSG/PM combination treatment for VL is toxic, painful, and require hospitalization and daily injections. There is an urgent need for improved treatments that are safe, effective, and appropriate for use in remote areas. An open-label, phase III, randomized, controlled, parallel arm, multicenter, non-inferiority clinical study was conducted in Ethiopia, Kenya, Sudan and Uganda to compare the efficacy of a combination regimen of paromomycin (PM) (20 mg/kg/d) and miltefosine (MF) (allometric dosage) for 14 days with the current 17-day standard of care sodium stibogluconate (SSG) (20 mg/kg/d) and PM (15 mg/kg/d), for the treatment of adult and paediatric patients with primary VL in Eastern Africa. The primary endpoint was the definitive cure at 6 months follow-up and secondary endpoints included initial and probable cure at day 28 and day 56, safety, pharmacokinetic (MF and PM) and pharmacodynamic assessments. A total of 439 patients,

predominantly male (80%), from 4 to 50 years old were recruited over a period of 29 months. A similar proportion of patients in the PM/MF and the SSG/PM arms achieved definitive cure at 6 months follow-up in the primary efficacy mITT set (91.2% and 91.8%, respectively). Non-inferiority was not demonstrated in the mITT population (the upper limit of the 97.5% CI of 7.4% exceeded slightly the non-inferiority margin of 7%), however was demonstrated in the per protocol set, with 92% in PM/MF 14D arm compared to 91.7% in the SSG/PM arm. In addition, the frequency of PKDL in patients from Ethiopia and Sudan was significantly higher in the SSG/PM arm (20.9%) than in the PM/MF arm (4.4%). The majority of adverse drug reactions (ADRs) were mild and moderate. The most common expected ADRs in the treatment arms were vomiting related to MF, injection site pain related to PM, and hypoacusis related to PM. ADRs suggesting cardiac toxicity related to SSG were reported in 6.5% of patients in the SSG/PM arm. A total of 18 serious adverse events (SAEs) were reported in 13 patients, 4 of them being considered related to the study drugs. The fatality rate in the trial was 0.9%, with one death being related to a study drug (cardiotoxicity related to SSG). The results of this study demonstrate that the 14 day PM and MF regimen achieved a clinically meaningful rate of cure with very similar efficacy to the standard of care (SSG/PM) in adult and paediatric VL patients. This new treatment was generally well tolerated, with ADRs as expected based on the known safety profiles of the drugs. It is more patient-friendly, since there is one less painful injection each day and the overall treatment duration is reduced by 3 days, it is associated with a lower incidence of PKDL, it has no risk of SSG-associated life threating cardiotoxicity, and it offers an alternative treatment regimen for patients with VL in Eastern Africa.

Keywords VISCERAL LEISHMANIASIS; AFRICA; CLINICAL TRIAL; PAROMOMYCIN; MILTEFOSINA

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O15-04: CUTANEOUS LEISHMANIASIS PATIENT CHARACTERISTICS AND TREATMENT OUTCOMES, QUETTA, PAKISTAN 2014-2021

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Cutaneous leishmaniasis (CL) is highly endemic in Pakistan, and causes a large public health burden, with an estimated 50-100,000 new cases annually. The most affected provinces are Balochistan and Khyber Pakhtunkhwa, where *Leishmania tropica* is the predominant species, transmitted by phlebotomine sandflies. At the location of the sandfly bite, a nodule appears, which typically develops into an ulcerating wound with secondary infections. These disfiguring wounds and scars often lead to stigmatization and (psycho-)social problems. Pentavalent antimonial drugs, the mainstay treatment is scarcely available in public hospitals in Pakistan. In the private health sector, if the drugs are available, it is provided at high costs, often resulting in sub-standard dosing. Since 2008, Médecins Sans Frontières (MSF) supports the Ministry of Health with specialised CL clinics providing free CL diagnosis and treatment services in Khyber Pakhtunkhwa and Balochistan. In Quetta, Balochistan, MSF have treated more than 26,000 CL patients in these 14 years. An electronic patient line lists with patient characteristics, treatment and outcomes, have been maintained since 2014. The aim of the retrospective observational study was to describe patient characteristics, analyse trends over the years, and to identify risk factors for negative treatment outcomes, i.e. treatment failure or relapse in CL patients treated with the meglumine antimoniate. Retrospective analyses were

performed of the CL patients cohort treated between 2014 and 2021. The database consisted of key dates, demographic, diagnostic and clinical characteristics of patients, treatment regime, initial treatment outcome, and follow-up outcome. The data were analysed by descriptive statistics and by logistic regressions with Wald statistics using chi square and Fisher's exact test for statistical significance difference. Variables were dichotomised in 'initial response' and 'no response' to treatment at the end of treatment, and 'final cure' and 'treatment failure' or 'relapse' at follow-up; six weeks after discharge. Of 19,600 patients with complete records, 47.2% were female, median age was ten years, 51.1% had facial lesions, and male patients presented with larger lesions. The proportion of patients receiving systemic treatment with intramuscular injections vs. local intralesional injections increased from 17.6% in 2014 to 49.0% in 2020. Poor initial response rate was 4.2%. Children <16years had almost 3-fold higher odds of poor initial response (OR 2.77 (95%CI 1.96-3.92). Treatment failure rate was 5.8% and relapse rate 0.9%. Logistic regression showed that being female, age <16years, lesion duration two months or shorter, lesion size less than five cm, facial lesions, and high parasitaemia, were associated with treatment failure. This is the first analysis conducted on such a large cohort of CL patients who received pentavalent antimonial treatment. An increase in patients requiring systemic treatment could indicate that patients come with more severe and larger lesions, which cannot be treated with intralesional injections. The insights gained from this research could be used to improve the treatment algorithms and to contribute to the CL national protocol in Pakistan. Further research is required to better understand the risk factors for failure and to develop appropriate preventive control measures against cutaneous leishmaniasis.

Keywords MEGLUMINE ANTIMONIATE; EPIDEMIOLOGY; SYSTEMIC TREATMENT

O15-05: EFFICACY AND SAFETY OF SHORT COURSE COMBINATION REGIMENS OF LIPOSOMAL AMPHOTERICIN B AND MILTEFOSINE FOR TREATMENT OF PKDL IN THE INDIAN SUBCONTINENT

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In the Indian subcontinent (ISC) all patients with post-kala-azar dermal leishmaniasis (PKDL) are treated, irrespective of time since visceral leishmaniasis (VL), previous VL treatment, duration of rash, or clinical presentation. While treatment may be indicated for clinical reasons, it is known that patients with PKDL play an important role in VL transmission and patients with chronic PKDL have been implicated in major VL outbreaks. This is crucially important for current VL elimination efforts in the ISC. Currently available PKDL treatments have limitations. The long 12-week regimen of first-line therapy miltefosine potentially impacts adherence to treatment and requires long-term contraception for women of childbearing potential. Recently, concerns have been raised over the risk of eye complications associated with this long treatment duration. Given the lack of quality evidence surrounding treatment of PKDL patients in the ISC, we conducted a randomized non-comparative clinical study to assess the safety and efficacy of two short duration treatment modalities. Male and female patients aged 6 to 60 years with a confirmed diagnosis of PKDL were invited to participate in the study. Patients fulfilling inclusion and exclusion criteria were randomly assigned to one of 2 treatment arms: AmBisome®

monotherapy (5 x 4 mg/kg IV given twice per week at a total dose of 20 mg/kg) or a combination of AmBisome® (5 x 4 mg/kg IV given twice per week at a total dose of 20 mg/kg) plus an allometric oral dose of miltefosine for three weeks. Following treatment, patients were followed-up for 2 years. The primary efficacy endpoint was based on definitive cure at 12 months according to clinical criteria: complete resolution of papular and nodular lesions (flattening of 100% of lesions) and significant improvement (> 80% re-pigmentation) of macular lesions by 12 months after the end of treatment. The secondary efficacy endpoint was based on overall clinical improvement assessed by clinician. In addition, PK analysis in skin and blood, host immune response and parasite clearance by microscopy and PCR were performed. 126 of the 142 patients screened met the inclusion criteria and were enrolled in the trial, 63 per study arm. Patients had a median age of 25 years, 56.35% were male. Patient characteristics in the two study arms were homogeneous at baseline. Treatment was well-tolerated overall; 1 serious adverse event with a fatal outcome was observed, which was considered unrelated to the study drug; the majority of adverse drug reactions were mild and moderate. The most commonly observed adverse events were vomiting, back pain, pyrexia, nausea, hypersensitivity and hypokalaemia. Efficacy results, as well as pharmacodynamics and immunology results and their relationship with clinical outcome, are under analysis and will be presented during the conference. Pharmacokinetics results will be presented in a separate abstract. The results of this study will be presented to the National Control Program and other stakeholders in the region. If the outcome shows satisfactory efficacy and good safety profiles for the new regimens, this evidence (together with data from other studies) could support policy changes for PKDL treatment in the region.

Keywords POST KALA AZAR DERMAL LEISHMANIASIS; INDIA; BANGLADESH; AMPHOTERICIN B; MILTEFOSINE

Financing The French Development Agency, France; the Dutch Ministry of Foreign Affairs (DGIS), the Netherlands; the World Health Organization–Special Programme for Research and Training in Tropical Diseases; UK aid, UK; Médecins sans Frontières International; the Swiss Agency for Development and Cooperation (SDC), Switzerland; for supporting its overall mission

O15-06: A MULTI-FUNCTIONAL DRUG RELEASE SYSTEM FOR SYNERGISTIC HEALING OF DERMAL ULCERS CAUSED BY CUTANEOUS LEISHMANIASIS

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Cutaneous leishmanaisis (CL), a neglected parasitic skin disease, lacks effective, affordable, and easy-to-use treatment options. Despite the pathophysiology of CL infection and the known benefits of wound occlusion, no front line treatment for CL in the Americas is currently delivered to the lesion site. Electrospun fibers have been used extensively as drug delivery systems and to promote wound healing for other skin pathologies. In this study, we report the fabrication of a multi-compartment topical patch for treatment of CL using electrospinning and electrospraying techniques. This multi-drug scaffold leverages material properties to co-encapsulate an antileishmanial drug (miltefosine), wound healing compound (allantoin), and antibiotic (gentamicin) for topical, spatiotemporal delivery to the lesion site. Briefly, two compartments are composed of a non-woven fibrous scaffold, whose core-shell fibers consist of a poly(ε-caprolactone) (PCL) sheath and a hyaluronic acid (HA) core, and the third compartment consists of enmeshed PCL particles. Miltefosine was encapsulated in the fiber's PCL sheath, allantoin was encapsulated in the fiber's HA core, and gentamicin was encapsulated in the PCL particles. *In vitro* release testing in cell culture media confirmed temporal drug release of gentamicin, followed by miltefosine, and ending with allantoin. This is in agreement with the desired physiological sequence to optimize dermal wound healing with minimal scarring. Patch release kinetics *in vitro* demonstrated feasibility of extended drug delivery from a single patch construct up to two weeks: by day 14, approximately 80% of the gentamicin, 55% of the milfefosine, and 25% of the allantoin was released. Constructs were also evaluated for

biocompatibility in a subcutaneous implantation mouse model. Histological analysis revealed extensive cellularization with limited immune response in the surrounding dermis tissue. Subsequent environmental stress testing, utilizing both packaged and unpacked patches, demonstrated that this design may be durable and feasible for use in hot and humid environmental conditions in rural endemic areas of transmission. No significant loss in scaffold mass or loaded drug content was recorded over three weeks inside a standard Environmental Testing Chamber. Combined, these preliminary findings suggest that such a patch design is promising for improved therapeutic treatment of ulcerative CL. This work may have implications for CL control in rural endemic areas where existing CL treatments are otherwise of limited feasibility and acceptance. More broadly, this work indicates a potential drug delivery platform and approach for tackling neglected global health challenges that can be applied to other debilitating tropical skin diseases.

Keywords TOPICAL DRUG DELIVERY; ELECTROSPINNING; WOUND HEALING; CUTANEOUS LEISHMANIASIS

O22-01: THE DEVELOPMENT OF AN ORAL OLEYLPHOSPHOCHOLINE TREATMENT FOR CUTANEOUS LEISHMANIASIS

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With an estimated 0.7 to 1 million new infections a year globally, cutaneous leishmaniasis (CL) is the most prevalent form of leishmaniasis; it clinically manifests as a variety of skin lesions ranging from closed nodules, to plaques and ulcers. Currently recommended drugs have proved to be clinically unsatisfactory indicating the urgent need for novel safe and efficacious drugs. Oleylphosphocholine, an alkylphoshoplipid structurally similar to miltefosine, demonstrated potent activity against *Leishmania* species causing visceral leishmaniasis (VL) both *in vitro* and *in vivo*. Given the discrepancies between the target product profiles of VL and CL, we here report the *in vitro* and *in vivo* efficacy of orally administered oleylphosphocholine-based formulations (two with a fast-release and two with a slow release profile) against CL-causing *Leishmania* species. The antileishmanial activities of OLPC and miltefosine were evaluated against intracellular amastigotes of six *Leishmania* species (*L. major*, *L. tropica*, *L. aethiopica*, *L. mexicana*, *L. braziliensis*, *L. panamensis*). Following promising results, the *in vivo* efficacies of both drugs were investigated in two stages. First, the performance of the efficacious dose for OLPC for VL was evaluated using an experimental CL model. Secondly, the antileishmanial activity of various formulations of OLPC with diverse release profiles was investigated alongside a dose response using bioluminescent *L. major* parasites. Tissue concentrations in skin of OLPC were determined using LC-MS/MS. The *in*

vitro activities of OLPC against CL-causing species ranged from 0.74 to 31.06 uM and are similar to those obtained for miltefosine. In the experimental CL models, OLPC administered orally at a dose of 35 mg/kg once daily for ten days was able to significantly reduce the lesion size to a similar extend as the positive control (paromomycin sulphate, ip, 50 mg/kg/day – repeated-measures ANOVA, post-hoc Tukey, $p < 0.05$). In contrast, the administration of miltefosine (same dose and regimen as OLPC) only resulted in a halt of the lesion size progression but was unable to decrease the lesion diameter. The second *in vivo* study was able to confirm these results and demonstrated a superior activity of the fast- (OLPC with lactose or cellulose carrier) over the slow-release (OLPC absorbed into a diffusion-controlled silica carrier) test formulations as measured by a significantly greater bioluminescence signal (~ parasite load) decrease when compared to the untreated controls. Extraction of the drugs from the infected skin site 24 hours after the oral administration of 1 dose (35 mg/kg) demonstrated higher concentrations of OLPC versus miltefosine (t-test). This difference was no longer present at the end of the 10-day treatment period even though OLPC blood concentrations at the end of treatment were 2-fold higher than for miltefosine. OLPC demonstrated potent activity in the intracellular macrophage model using a range of CL-causing species and was able to reduce the parasite load in an experimental *L. major* CL model after ten days of treatment. In a next step, the drug delivery profile into *Leishmania*-infected and uninfected mouse skin will be compared using skin microdialysis.

Keywords OLEYLPHOSPHOCHOLINE; MILTEFOSINE; CUTANEOUS LEISHMANIASIS; SKIN PHARMACOKINETICS

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O22-04: DEVELOPMENT OF A BIOCOMPATIBLE POLYMERIC CHITOSAN SYSTEM FOR THE RELEASE OF COMPOUNDS WITH LEISHMANICIDAL ACTIVITY

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Leishmaniasis is a tropical disease caused by parasites of the genus *Leishmania* spp; it presents different clinical manifestations, of which cutaneous leishmaniasis is the most common form, and it is endemic in more than 70 countries worldwide. Only four drugs are registered for the treatment of the disease, all of them associated with high toxicity. Recently, the *in vitro* and *in vivo* leishmanicidal activity of a mixture of the benzoic acid 2-(2,3-dihydro-4H-1-benzopyran-4-ylidene) hydrazide (TC2) and the compounds present in the plant extract *Sapindus saponaria* L (SS) were reported in other works. In the present study soft capsules of chitosan were developed incorporating TC2 and SS in 1:1 (CQ) ratio and these showed low cytotoxicity in U937 macrophages but moderate cytotoxicity in Detroit 551 fibroblast by MTT method and had a high antileishmanial activity against intracellular L. *braziliensis* amastigotes strain transfected with the green fluorescent protein (MHOM/CO/88/UA301-EGFP). Was possible to detect SS and TC2 using high performance liquid chromatography, and to quantify TC2. It was determined that the release kinetics of TC2 depends on the pH of the medium. An *ex vivo* model with Franz Cells and pig skin membranes were used and was possible to detected SS and TC2 in the detector chamber after one hour of essay and release the compounds in a sustained manner for 48 hours. Finally, CQ were effective to treat experimental cutaneous leishmaniasis induced with L. (V) *braziliensis* in hamster model. Each CQ developed in this study was properly loaded with 34 ± 5 µg of TC2 and SS, and when rubbed on the skin until was completely absorbed without any

trace or sticky and greasy skin. The application of 10 capsules was sufficient to cause an effect against intracellular amastigotes of *Leishmania spp*. It was determined in a hamster model of Leishmaniasis that after topical application of 40 mg of CQ in the ulcer, daily and for two months the effectiveness is greater than 80%. In conclusion, the CQ can release TC2 and SS, which diffuse through pig skin in less than one hour, and the concentration increases to generate a leishmanicidal effect as a cutaneous application for leishmaniasis.

Keywords CUTANEOUS LEISHMANIASIS; LEISHMANICIDAL ACTIVITY; DRUG RELEASE KINETICS; *IN VITRO; EX VIVO; IN VIVO*

O22-06: FORMULATION OF AMPHOTERICIN B IN PEGYLATED LIPOSOMES FOR IMPROVED TREATMENT OF CUTANEOUS LEISHMANIASIS BY PARENTERAL AND ORAL ROUTES

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Liposomal amphotericin B (AmB) or AmBisome® is the most effective and safer therapeutic agent for visceral leishmaniasis (VL), but its clinical efficacy is limited in cutaneous leishmaniasis (CL) and HIV/VL co-infection. The aim of this work was to develop a formulation of AmB in PEGylated liposomes and compare its efficacy to AmBisome® in a murine model of CL. In this work, a simple and original process has been developed for the encapsulation of AmB in pre-formed empty liposomes. Formulations of AmB in conventional and PEGylated liposomes were characterized for particle size and morphology, drug encapsulation efficiency and aggregation state. The conventional and PEGylated formulations showed vesicles with 100-130 nm diameter and low polydispersity (PI<0.3), incorporating more than 95% of AmB under the non-aggregated form. Those were compared to AmBisome® in *Leishmania amazonensis*-infected BALB/c mice for their effects on the lesion size growth and parasite load. In a first set of experiments, the formulations were given every 4 days (5 mg/kg of AmB), either by IP or IV route. Following 7 doses of parenteral treatment in murine CL, the PEGylated formulation of AmB significantly reduced the lesion size growth and parasite load, in comparison to control groups (saline or empty

liposomes), in contrast to conventional liposomal AmB and AmBisome®. The same profile of parasite suppression was observed in the spleen of animals, with significant parasite suppression only in the group that received PEGylated liposomal AmB formulation. In a second step, infected mice were also treated with 10 doses of the PEGylated AmB formulation (5 mg/kg of AmB) given at 2-day intervals, by oral and IP routes. The PEGylated formulation given orally promoted significant reductions of the lesion size growth and the parasite load, to comparable levels as those achieved by IP AmBisome®. Evaluation of the parasite load in the spleen showed significant parasite suppression only in the groups that received the formulations by IP route. In the latter experiment, the groups that received liposomal AmB formulations were further evaluated regarding serum markers of the renal (urea, creatinine) and hepatic functions (ALT and ALP), in comparison to the control group. Only urea showed significant change, with significantly increased levels in AmBisome® (IP) and PEG-LAmB (IP) groups, demonstrating a reduced renal toxicity of the oral formulation. This work reports for the first time that PEGylated liposomal AmB can improve treatment of experimental cutaneous leishmaniasis by both parenteral and oral routes, in comparison to AmBisome®. The formulation AmB in pegylated liposomes is a promising candidate drug for the treatment of CL.

Keywords LIPOSOMES; AMPHOTERICIN B; ORAL ROUTE; PEGYLATION; CUTANEOUS LEISHMANIASIS

Financing CNPq (Brazil), Chaire Jean d'Alembert/Chaire d'Excellence DIM1Health (France)

O29-01: miR-548d-3p ALTERS PARASITE GROWTH IN *Leishmania (Leishmania) infantum* INFECTION

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Visceral leishmaniasis caused by *Leishmania (Leishmania) infantum* in Latin America may evolve to lethality if not treated. Infection development is related to host immune response but also to changes in the gene expression related to immune and inflammatory responses. *Leishmania* infection modulates host gene expression including miRNA. The mechanisms and the respective relationship with parasite pathogenesis are not well understood, especially modulation of miRNAs that may influence *Leishmania* infection and pathogenesis. In the present study, searching the biological significance of alterations of gene expression upon infection, miRNAs associated to autoimmune and inflammatory pathways were quantified using qPCR arrays in *L. (L.) infantum* promastigote-infected human monocytic THP-1 cells in vitro and in plasma from patients with visceral leishmaniasis. We identified some differentially expressed miRNAs in infected THP-1 cells when compared with non-infected cells. Then these miRNAs were submitted to *In Silico* analysis revealing different targets in TGF-β and

glucose metabolic pathways, inflammation, apoptosis, and cell signaling. We identified in active visceral leishmaniasis plasma differentially expressed miRNAs compared with endemic healthy controls. *In Silico* analysis of these miRNA showed different predicted targets, highlighting targets in the TGF-β, TLR4, IGF-I, HIF1 pathways. Some of the differentially expressed miRNAs during THP-1 in vitro infection were also found in plasma from active visceral leishmaniasis patients, including miR-548d-3p upregulated in both approaches. To evaluate the potential biological effect of miR-548d-3p, we proceed with transient transfection of *L. (L.) infantum* infected- THP-1 cells using miR-548d-3p inhibitor. Inhibition of miR-548d-3p enhanced the parasite growth early after infection. As miR-548d-3p targets pathways related to inflammation, MCP1/CCL2, RANTES/CCL5, and IP10/CXCL10, IL-8/CXCL8, MIG/CXCL9 altered production was evaluated upon inhibition. Our results suggest that modulation of miR-548d-3p by *L. (L.) infantum* may modulate inflammation and decrease parasitism. Besides participation in pathogenesis, miR-548d-3p may cconstitute a target, candidate and prognostic biomarker.

Keywords *Leishmania (Leishmania) infantum*; microRNA; VISCERAL LEISHMANIASIS; THP-1 CELLS; PATHOGENESIS; INFLAMMATION

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O29-02: MICRONEEDLE-BASED DELIVERY OF FUNGIZONE/AMPHOTERICIN B IS EFFECTIVE FOR THE TREATMENT OF AMERICAN CUTANEOUS LEISHMANIASIS

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Cutaneous leishmaniasis (CL), including those cases caused by *Leishmania mexicana,* affects 12 million patients annually in the form of disfiguring skin lesions. These lesions often require medical intervention and can lead to lasting stigma and marginalization for patients due to scarring. Amphotericin B is an especially potent & widely accessible FDA-approved drug against CL; however, it is reserved for severe cases due to its narrow therapeutic range and potential for severe side effects such as liver and kidney toxicity. While lipid formulations of Amphotericin B such as AmBisome have lessened toxicity concerns, AmBisome is not universally affordable. An alternative solution to improve patient tolerance of traditional Amphotericin B is drug delivery through microneedles. Microneedles – an array of short needles – can deliver medication directly to the CL lesion without exposing the patient to systemic toxicity, thereby improving safety and efficacy of amphotericin treatments relative to IV administration. These needles are designed to shallowly penetrate the skin over a prescribed surface area, allowing the stratum corneum to be bypassed and for drug to be injected into the lesion in a noninvasive and painless manner. Additionally, these devices can be used without specialized

medical training or facilities. Our study evaluated a novel hollow microneedle device to deliver commercially formulated Fungizone Amphotericin B for treatment of *L. mexicana* cutaneous lesions in a susceptible mouse model. The microneedles were situated in a 3-by-3 array, with the center of each needle spaced 3.5 mm from the next. These microneedles were shown using scanning electron microscopy to exhibit sharp tips, heights of 1 mm, outer diameters of 250 µm, and inner diameters of 150 µm. Optical coherence tomography demonstrated the penetration capabilities of the microneedle device. In a BALB/c mouse model infected intradermally with *L. mexicana*, daily use of these microneedles to deliver Fungizone Amphotericin B directly into the CL lesions over 10 days resulted in lower lesion size relative the placebo control group. Five weeks after the end of microneedle treatment, draining lymph nodes from the experimental group still showed significantly lower parasite burden relative to the placebo control group. At this timepoint, draining lymph nodes of the treatment group also showed significantly higher levels of Th17 cells in the drug group relative to the control group via flow cytometry. Previous *in* vivo studies on CL caused by *L. mexicana* and *L. panamensis* showed a positive correlation between Th17 cells and inflammasome activation and an inverse correlation to amastigote number, which could explain our findings. These promising results build upon previous studies showing the potential utility of microneedle treatments for CL and represent the first use of hollow microneedles for CL treatment. Future work should include further characterizing their safety and efficacy as well as investigating broader applications with other CL drugs and immunotherapy strategies. These microneedle devices ultimately may offer substantial benefits to patients and healthcare providers alike and lessen the burden of CL in affected communities due to their ease of use and demonstrated efficacy.

Keywords MICRONEEDLES; CUTANEOUS LEISHMANIASIS; DRUG DELIVERY; AMPHOTERICIN B; *IN VIVO*

O29-03: SAFETY AND EFFICACY OF TWO NEW TREATMENTS FOR PATIENTS WITH POST-KALA-AZAR DERMAL LEISHMANIASIS: A RANDOMIZED PARALLEL ARM OPEN LABEL STUDY IN SUDAN

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Post-kala-azar dermal leishmaniasis is a skin rash that may occur months or even years after a successful treatment of visceral leishmaniasis, and presents as macular, nodular or papular lesions. Despite not being a life-threatening condition, PKDL is difficult to treat, requiring longer treatment duration than VL. In Sudan, treatment is recommended only for chronic or severe cases, consisting of sodium stibogluconate (SSG) for 40-60 days (possibly extending up to 90 days), SSG/paromomycin (PM) combination or Liposomal Amphotericin B (LAmB) (total dose of 50mg/Kg), all requiring prolonged hospitalization. The long duration of SSG therapy may be associated with risk of cardiotoxicity, pancreatitis and hepatitis, which is not justifiable for a patient who is on average not ill. A safe treatment is needed that can be administered as an out-patient, or require shorter hospitalization period. Our strategy was to combine one parenteral drug (LAmB or paromomycin), administered for a short period, with an oral drug (miltefosine), administered for a longer duration, to achieve satisfactory efficacy. This was an open label, randomized non-comparative phase II clinical trial to assess the safety and efficacy of a combination of paromomycin (20 mg/kg/d) IM for 14 days with oral miltefosine (allometric dosing) for 42 days (PM/MF), and a combination of LAmB (20 mg/kg total dose) IV over 7 days with oral miltefosine for 28 days (allometric dosing) (LAmB/MF). The primary objective was to assess the safety and efficacy of

the two combination regimens. The primary efficacy endpoint was definitive cure at 12 months after treatment onset, defined as clinical cure (100% lesion resolution) and no additional PKDL treatment between end of therapy and 12 months follow-up assessment. Safety was assessed through the frequency of SAEs, frequency and severity of AEs that lead to treatment discontinuation, and frequency and severity of all AEs from treatment onset through 12-month follow up period. Secondary objectives were to assess the pharmacokinetics of the study drugs in blood and skin, parasite clearance and immunological parameters prior, during and after treatment. A total of 110 patients were enrolled in the trial, 55 per study arm. The study population was homogeneous, with similar baseline characteristics (age, sex, lesion type and density, etc). Majority were children (64% less than 12y) and the median duration of PKDL was 18 months. Efficacy was 98.2% (95% CI, 90.3-100) for patients in PM/MF arm and 80% (95% CI, 70.2-91.9) for patients in LAmB/MF arm (mITT). There was no SAE in the trial. Two patients had treatment discontinued: one case of hypersensitivity after LAmB administration and one case of acute kidney injury related to PM/MF. Vomiting, mostly mild, was the most common adverse event (44% in MF/AmB arm and 20% in MF/PM arm). Patients in the LAmB/MF arm had higher adverse drug reactions than MF/PM arm (51% vs 24%). Grade 3 or 4 AEs (hypokalaemia and increased transaminases) were reported in 14.5% of patients in the LAmB/MF arm.
Based on the efficacy and safety findings, PM/MF seems to be a better option to treat patients with PKDL in Sudan.

Keywords PKDL; SSG; PAROMOMYCIN; MILTEFOSINE; SUDAN

O29-04: PROTEIN KINASES INVOLVED IN CELL CYCLE PROGRESSION VALIDATED AS DRUG TARGET IN *Leishmania*

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Globally distributed and closely related to poverty, the leishmaniases are neglected tropical diseases which control relies primarily on chemotherapy. Nevertheless, current therapies have severe shortcomings, highlighting an urgent need for innovative safe and efficacious treatments. We use chemical genetic approaches to validate protein kinases as drug targets and study their biological role in *Leishmania*. To this end we used CRISPR-Cas9 to perform precision editing of the *L. mexicana* genome to generate analogue sensitive mutants suitable for chemical genetic inhibition. For the kinetochore protein kinase KKT2, the cyclin-dependent kinase CRK9 and a CMGC family protein kinase MSK, a replacement of the bulky gatekeeper methionine residue with a glycine in the ATP-binding site makes the enzymes sensitive to the bulky inhibitor 1NM-PP1. For the kinetochore protein kinases CLK1 and CLK2 (also known as KKT10 and KKT19, respectively) replacement of a cysteine near to the ATP-binding domain prevents binding of the covalent Michael-acceptor in the inhibitor AB1 (Novartis), validating the specificity of this compound against CLK1/CLK2. The chemical validation demonstrated that these protein kinases are essential for the promastigote and intracellular amastigote stages of the parasite. The specific inhibition of CLK1/CLK2, KKT2 and MSK caused a cell cycle arrest in G2/M stage of the promastigote. A further investigation, by fluorescence microscopy labelling the mitotic spindle, revealed that KKT2 inhibition is followed by a significant accumulation of cells in early mitosis, where mitotic spindle coordination in the nucleus failed. Furthermore, it

was observed that MSK inhibition also impaired chromosome segregation, but the cell body development reaches a more advanced stage, suggesting MSK activity is required later in mitosis than KKT2. In addition, CLK1/CLK2 inhibition doesn't affect the coordination of the mitotic spindle, but it blocks cell cycle progression in cytokinesis accumulating multinuclear cells. These studies bring new insights into the essential biological process of cell division in *Leishmania* and provide a source of new potential therapeutic targets.

Keywords DRUG TARGET VALIDATION; *LEISHMANIA*; PROTEIN KINASES; CHEMICAL GENETIC APPROACHES

Funding GCRF, A Global Network for Neglected Tropical Diseases

O29-05: DEVELOPMENT AND EFFICACY TEST OF A HYDROGEL FOR THE THERMOTHERAPY OF CUTANEOUS LEISHMANIASIS

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Leishmaniasis is a tropical disease caused by parasites of the genus *Leishmania* spp; it presents different clinical manifestations, of which cutaneous leishmaniasis is the most common form, and it is endemic in more than 70 countries worldwide. Available treatments are limited in number and efficacy, and have disadvantages such as prolonged administration, high doses, poorly tolerated systemic route of administration, and toxic effects. Therefore, it is necessary to continue exploring therapeutic alternatives such as thermotherapy that have been shown to be effective and safe. A hydrogel-type biomaterial was developed, and preclinical trials were carried out. A biomaterial was developed polymerizing acrylamide and acrylic acid, via free radicals, and water absorption was confirmed by swelling tests. Cytotoxicity assays were performed by MTT method, using U937 macrophages and Detroit 551 fibroblast. K-type thermocouples were used to determine the temperature profile on pig ear skin after applying the hot hydrogel. *In vivo* activity of hydrogel was evaluated in 6-week-old hamsters experimentally infected on the back skin with *L. braziliensis*. Once the typical skin lesion developed, general anesthesia was applied and they were treated topically with the hot hydrogel, in therapeutic schemes of one application, every other day, making 3 total applications. General clinical and lesion follow-up was carried out for three months after post-treatment. Within the preclinical studies, an observational study was carried out with 3 patients. The hydrogel was applied with previous topical anesthesia and the same scheme used in the *in vivo* test. The patients included in the study were patients who were diagnosed with uncomplicated cutaneous leishmaniasis and who, due to difficulties in accessing follow-up, were offered an alternative treatment by thermotherapy within the framework of the Compassionate Use Treatment Program. All participants signed the

Informed Consent. The hydrogels absorbed water up to 100 times their dry weight. It was possible to characterize that when the hydrogel is introduced into boiling water for 1 minute and subsequently applied to the skin of the pig's ear, the temperature of the skin increases to 52°C at a depth of 3 mm and remains oscillating for above 48°C for 4 minutes. Cytotoxicity showed cell viability of 92.5 ± 1.8% and 100% in macrophages U937 and Detroit 551 fibroblast, respectively. In 4 of 6 animals there was 100% improvement at two weeks post-treatment and at 12 weeks post-treatment, 5 animals were cured and the remaining one showed improvement of the lesion with a reduction in size by 89%. In the observational trial, the treated patients began to show improvement on the third day of treatment, when the induration began to decrease. No adverse reaction was recorded. After two weeks healing of the lesions was observed and after a month they were completely healed. None of the lesions reactivated and they remain lesion-free up to 1-year post-treatment. In conclusion, a promising hydrogel medical device was developed for the treatment of uncomplicated cutaneous leishmaniasis, which was shown to be safe and effective in preclinical studies.

Keywords CUTANEOUS LEISHMANIASIS; THERMOTHERAPY; CYTOTOXICITY; BIOMATERIALS; *IN VIVO* EFFICACY; PRECLINICAL TRIALS

O29-06: EFFECT ON THE INTERACTION OF MACROPHAGE AND LEISHMANIA IN THE THERAPEUTIC EFFECTIVENESS OF A NOVEL MIXTURE OF TRITERPENIC SAPONINS AND CHROMANIUM HYDRAZONE

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Leishmaniasis is a worldwide-expanded zoonotic disease caused by the infection with digenetic parasites of the genus *Leishmania*. Current chemotherapy agents for leishmaniasis are either toxic, expensive, or both, interfering with the effective treatment of people around the world, and therefore more and better antileishmanial drugs are currently needed. In previous research, our group identified SS saponins with potential leishmanicidal activity from crude extracts of *Sapindus saponaria*, as well as compounds of the chromane hydrazone called TC1 and TC2. As an approach to the mode of action, in addition to the cytotoxic and antileishmanial activity of the compounds, individually or in combination, the effect of the compounds on mitochondria, phagolysosomes, ATP levels, production reactive oxygen species, and protease activity was evaluated using fluorometry, microscopy, flow cytometry, enzymology and metabolomic methods. All products, chromane hydrazones, and triterpenic saponin and their mixtures showed differential and selective toxicity according to cell type. In vitro antiparasitic assays showed moderate activity against Leishmania species with values < 20 µg/mL, but was lower when the molecules were added in combination. This effect was associated with changes in mitochondrial activity, inducing a reduction in ATP availability, structural changes in membranes, and overproduction of reactive oxygen species. In addition, the cell-parasite relationship was also modified with

morphological changes in phagolysosomes, a decrease in protease activity in the parasite, changes in the phenotype of infected macrophages, and alteration in the tryptophan pathway, affecting Leishmania survival and suggesting its reduced reproduction and infectivity. The present study shows the reason for the potentiation of antileishmanial activity shown in vivo trying to explain the role of each component for the healing process.

Keywords Chromane hydrazone; Leishmania; Sapindus saponaria; Host parasite interaction, drug effectiveness

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O36-01: AN EFFECTIVE NASAL NANOPARTICULATE IMMUNO-TREATMENT AGAINST LEISHMANIASIS IN DOGS

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Leishmaniasis are zoonosis caused by *Leishmania* sp. parasites and transmitted to human, dogs, and rodents by *Phlebotomous* sand fly. Depending on the parasite strain, the most common diseases' outcome are cutaneous incurable ulcers (CL) and/or visceral infection (VL). *Leishmania infantum* infects both dogs and humans. The most used drugs in the treatment of leishmaniases in dogs are pentavalent antimonials such as sodium stibogluconate or Glucantime, paromomycin and miltefosine, or allopurinol. These treatments have many pitfalls such as high toxicity and side effects, decreasing efficacy due to resistance emergency and are pricey. To overcome these issues, here we propose a novel immuno-treatment based on maltodextrin-nanoparticles incorporating *L. infantum* antigens (NP-Linf). Thirty *L. infantum* positive dogs from an endemic region of North-eastern Brazil at stage 2 of infection (*Leishvet.org*) were recruited. We compared the efficiency of a Miltefosine treatment (10 dogs, 2 mg/kg, every day for 28 days) to nasal administrations of NP-Linf (10 dogs, 100µg at two weeks interval) and to the combination of the two treatments (10 dogs). The animals were evaluated for a period of 180 days, and they remained at home with their owners during the whole study period. The efficacy of each treatment against CL and VL was assessed all along the study by the microscopy of bone marrows and skins, as well as the IFAT evaluation (cut off = 1/40 dilution) at T0 and T180 days post treatment. NP-Linf intranasal administrations were well tolerated by dogs and no adverse effect occurred while in the Miltefosine group 4 dogs died during the treatment. Twelve weeks after the beginning of the study, no parasite was detected in the skin of dogs treated with NP-Linf. On the contrary, in the Miltefosine group 2 dogs

still had CL. IFAT analysis revealed that vaccinated dogs had a high decrease of antibodies in the serum. Altogether the results suggest higher efficacy of the nanoparticle-based immunotherapy in reducing the number of administrations (2 vs 28) without any side effect. These results suggest that further studies in order to develop this treatment can be envisaged to cure infected dogs. Further studies in term of following the cell mediated immunity induced by the immuno-treatment are planned.

Keywords LEISHMANIASIS; IMMUNOTHERAPEUTIC; DOGS; NASAL VACCINE

O36-03: SAFETY, PHARMACOKINETICS AND IMMUNE EFFECTS IN HEALTHY VOLUNTEERS OF CPG ODN D35, A TOLL-LIKE RECEPTOR 9 AGONIST THERAPEUTIC FOR LEISHMANIASIS

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Treatment of cutaneous leishmaniasis (CL) aims to increase the rate of complete cure, shorten time to heal, reduce scarring and transmission, and prevent the development of complicated forms, i.e., mucocutaneous leishmaniasis or leishmania recidivans. One approach to treatment is to eliminate most organisms by chemotherapy and then leave the host immune mechanisms to control the remaining parasites. Another approach under investigation would be to use immunotherapy to enhance the patient's immune response to foster parasite clearance. In severe/high risk cases, a combined treatment could be the best approach. CpG ODN D35 is a class A/D CpG oligonucleotide Toll-like receptor 9 (TLR-9) agonist. It is a short, single-stranded synthetic DNA molecule (20 bases in length) that contains un-methylated cytosine-guanine (CG) dinucleotide motifs that can be detected by TLR-9 on plasmacytoid dendritic cells (pDCs) in humans. In primates, CpG ODN D35 stimulates maturation and activation of pDCs and production of proinflammatory cytokines, such as *IFN-α* and *IFN-γ*, which are required for control of *Leishmania* infection. CpG ODN D35 has little or no effect on B cells and does not elicit the Th2 type immune responses associated with

other classes of CpG ODN. A first-in-human clinical study investigated the safety, pharmacokinetics, and immune effects of single ascending doses of CpG ODN D35 administered subcutaneously in healthy male participants aged between 18 and 50 years. The study consisted of 3 cohorts of 8 participants randomly (6:2) assigned to receive a single dose of CpG ODN D35 (7.5, 22.5 and 67.6 mg) or placebo. The primary endpoint was safety and tolerability, including injection site reactions assessment. Secondary endpoint explored drug pharmacokinetics and pharmacodynamic effects were investigated as exploratory endpoints by measuring key cytokines (*CXCL10*, *IFN-γ*, *IL-6* and *IL-13*) in serum before dosing and at 8h, 12h, 24h, 48h and 7 days post-dose. Overall, CpG ODN D35 was well tolerated; none of the dose-escalation stopping criteria were met. Injection site reactions, mainly redness and tenderness that ranked as mild or moderate, were observed and were all self-limited. Transient and dose-dependent shifts in the number of neutrophils, lymphocytes, monocytes and basophils, which were probably related to the pharmacological activity of the product, were observed but were not clinically significant. A dose-dependent increase of *CXCL10* concentrations in serum was observed from 24 h to 7 days post dose, with a peak at 48 h, with no significant increases in *TNFα*, *IL-6* or *IL-13*, which is consistent with the non-clinical data and expected pharmacological activity. The maximum concentration of drug in plasma was detected around 0.2 hours after injection of 22.5 and 67.5 mg doses, associated with a very short half-life in plasma. A multiple-ascending dose study is planned to confirm the safety, tolerability and cytokine and chemokine expression pattern, following CPG ODN D35 dosing in patients with uncomplicated CL. We hypothesize that CpG ODN D35, used in combination with an anti-*Leishmania* compound will improve the outcome in patients with CL, including complicated forms of the disease.

Keywords LEISHMANIASIS ; CpG ODN D35 ; SAFETY ; FIRST-IN-HUMAN ; CYTOKINES

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O36-04: FIRST IN HUMAN STUDY IN HEALTHY SUBJECTS TO ASSESS THE SAFETY, TOLERABILITY AND PHARMACOKINETICS OF DNDI-0690, A NOVEL ORAL DRUG CANDIDATE FOR LEISHMANIASIS

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Leishmaniasis is a complex vector-borne disease with 20 causative agents and various clinical manifestations. Visceral leishmaniasis (VL), a systemic disease that is fatal when left untreated, is caused by the protozoan parasites *Leishmania donovani* (anthroponotic) in Asia and Eastern Africa, and *Leishmania infantum* (zoonotic) in Latin America and the Mediterranean region. In patients with VL, the disease is insidious with development of splenomegaly, irregular fevers, anaemia, pancytopenia, weight loss and weakness, occurring progressively over weeks or months. For over eight decades, patients with VL have been treated primarily with pentavalent antimonials, which have severe side effects and prolonged treatment duration, often leading to poor patient compliance, resulting in a heavy burden to health care systems. DNDi, together with industrial partners, has created an unprecedented portfolio of new chemical entities (NCEs), with the aim of developing safe, oral treatments for leishmaniasis. One promising candidate, DNDI-0690, a 7-substituted nitroimidazooxazine, shows very potent antileishmanial activity *in vitro*, demonstrating broad spectrum activity against a range of *Leishmania* strains. Des-nitro analogues of DNDI-0690 have been shown to be inactive against *Leishmania in vitro*, confirming that the nitro (NO2) group is essential for DNDI-0690's cidal activity, and that activation via parasitic nitro-reductase 2 appears to be required. DNDI-0690 administered by oral route is efficacious in mouse and hamster models

of acute and chronic VL infection (*L. infantum* and *L. donovani*), and in cutaneous leishmaniasis murine models. Portfolio front-runner drug candidate DNDI-0690 is, therefore, being investigated as an oral treatment for leishmaniasis in humans. A first-in-human clinical trial investigated the safety, tolerability and pharmacokinetics of single doses of DNDI-0690 in healthy male and female participants. A total of 64 healthy human participants were successfully enrolled in this study over 8 cohorts. A single oral dose of DNDI-0690 capsules (ranging from 10 to 3600 mg) or matching placebo was administered to 8 subjects (randomized 6:2) in each cohort. Primary endpoint included safety and tolerability variables and secondary endpoint assessed pharmacokinetic variables calculated from plasma and urine concentrations. Overall, DNDI-0690 was found to be well tolerated with a limited number of adverse events, all mild or moderate in severity, the majority of which were unrelated to the study drug. Mild increases in creatinine were observed; these were considered probably related to an inhibition of tubular secretion of creatinine caused by DNDI-0690, as cystatin C values and other renal safety monitoring parameters remained unaffected. DNDI-0690 was absorbed with a median Tmax occurring between 1.5 and 4.0 hrs post-dose and had a mean half-life ranging from 4.5 to 12.1 hours across all regimens, with no significant impact of food or sex. The maximum concentration reached in the last cohort (given 3600 mg dose) was 3160 ng/mL, with a maximum area under the curve (AUC_{0-24}) of 42,600 ng.h/mL. A multiple ascending dose study in healthy volunteers is currently ongoing and will provide further information on the safety and pharmacokinetic properties of DNDI-0690. Exploration of the glomerular filtration rate following iohexol administration will confirm the functional mechanism of the observed increase in creatinine.

Keywords LEISHMANIASIS; NEW CHEMICAL ENTITIES; SAFETY; FIRST-IN-HUMAN; PHARMACOKINETICS

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O36-05: DNDI-6174: A PRECLINICAL CANDIDATE FROM A NOVEL CHEMICAL CLASS AND MECHANISM OF ACTION TO TACKLE LEISHMANIASIS

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Associated with poverty and classified as a neglected infectious disease, leishmaniasis is one of the diseases targeted in the United Nations Sustainable Development Goals agenda. This vector-borne disease, caused by more than twenty *Leishmania spp* can be anthroponotic as well as zoonotic, is endemic in three main regions (East Africa, Latin America, and South Asia), and can lead to various manifestations. Given this complexity and the fact that current treatments have significant drawbacks in terms of efficacy, safety, compliance, and suitability for use in the field, there is a critical need to strengthen the current portfolio of therapeutic options. Indeed, while the number and quality of oral new chemical entities (NCEs) currently in the translational phase is unprecedented, additional drug candidates that bring novelty would be welcome to circumvent the anticipated attrition rate and provide innovative treatments. A phenotypic

screen of 1.8 million molecules against kinetoplastid parasites (Peña, I., Pilar Manzano, M., Cantizani, J. *et al*. *Sci Rep* **5,** 8771 (2015)) identified one promising hit with a novel pyrrolopyrimidine chemotype. Following independent hit-to-lead and lead optimization efforts on the resulting chemical series 205/220, a research collaboration between non-profit, pharmaceutical, and academic organisations emerged and led to the discovery and candidate profiling of DNDI-6174. DNDI-6174 showed excellent *in vitro* efficacy against *Leishmania* lab strains and submicromolar potency was confirmed against many clinical isolates from various origins. *In vivo*, DNDI-6174 was shown to be highly effective against *L. donovani* and *L. infantum* infections in both mouse and hamster. Efficacious regimens ranged from 5 to 10 days at a daily dose of 12.5 to 25 mg/kg. Mechanism of action (MoA) studies (resistance generation, whole genome sequencing, and biochemistry inhibition assays) revealed that DNDI-6174 targets the Q_i site of cytochrome b, part of the *Leishmania* cytochrome bc1 complex (complex III). Given this MoA, the risk of mitochondrial toxicity in the human host has been ruled out through a cascade of *in vitro* assessments. *In vitro* profiling and oral administration to mice, rats, and dogs demonstrated that DNDI-6174 has very promising ADME properties, supporting further development. The safety profile for DNDI-6174 is promising, as demonstrated by the results of a safety pharmacology panel and assays, and a 14-day exploratory toxicity study in rat. Finally, with the perspective of potentially combining DNDI-6174 with another NCE, the preliminary drug-drug interaction (DDI) risk was assessed and found to be manageable. In summary, the profile of DNDI-6174 is consistent with the target candidate profile defined by DND*i* for visceral leishmaniasis. This makes it a promising candidate for a clinical trial application/investigational new drug-enabling package, with the potential to meet the corresponding target product profile. Belonging to a novel chemical class (pyrrolopyrimidine), with a new MoA (cytochrome bc1 complex inhibition) and an encouraging therapeutic index (>10), DNDI-6174 has the potential to become an important drug in the effort to eliminate leishmaniasis.

Keywords LEISHMANIASIS; NEW CHEMICAL ENTITIES; MECHANISM OF ACTION

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O36-06: IMMUNE STIMULATORY COMPPOUNDS FOR HOST-TARGETED THERAPIES IN INTRACELLULAR *Leishmania* INFECTION

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Immune stimulatory compounds capable of inducing a protective activation stage of macrophages represent a promising strategy for the treatment of leishmaniasis. Synthetic analogs derived from the phosphatidylinositol-b-anchor (PIb) of an immunostimulatory glycolipid molecule from the intestinal protozoan parasite *Entamoeba histolytica* (*Eh*) previously showed considerable immunotherapeutic effects against dermotropic and viscerotropic *Leishmania* (*L.*) ssp. *in vitro* and against cutaneous infection with *L. major in vivo*. Here we analyzed the treatment efficacy of *Leishmania*-infected primary human macrophages after stimulation with synthetic *Eh*PIb compounds and evaluated their supportive effect by combined treatment strategies with reference drugs. Human macrophages were generated from $CD14^+$ monocytes from healthy male and female blood donors (buffy coat), infected with dermotropic *L. major* or viscerotropic *L. infantum* and treated with synthetic *Eh*PIb compounds in a single or combined treatment regimen 24 hpi. Treatment efficacy (host cell viability, infection rate, parasite burden) was analyzed using a high content screening assay, based on a parasitic 90 kDA heat shock protein-specific staining, enabling the detection of several *Leishmania* species. Treatment-specific immune response was investigated using multiplex cytokine bead assay. Preliminary data revealed, that three (Eh-1, Eh-5, Eh-6) out of six *Eh*PIb compounds exhibited considerable anti-leishmanial activity against both *L. major* and *L. infantum* infected human macrophages, without negatively affecting host cell viability. Compound Eh-1 was analyzed in more detail

and, interestingly, indicates a sex-related difference in the therapeutical outcome against *L. major* infection, as a significant reduction of the infection rate was observed in female but not in male macrophages. Moreover, the addition of Eh-1 appears to enhance the efficacy of Amphotericin compared to use of this reference drug alone. Analysis of immune profiles after treatment with Eh-1 alone showed a decrease of Th2-related cytokines and chemokines (IL-10, Arginase, CCL17) compared to *L. major*-infected but untreated macrophages and a slight increase of Th1-related IL-1 was induced post treatment, whereas IP-10 tended to decrease. So far, the use of host-targeting synthetic *Eh*PIb compounds, either alone or in combined treatment regimens with antiparasitic drugs, show a certain potential for the treatment of cutaneous and visceral leishmaniasis and therefore might improve the current unsatisfactory status of chemotherapy against this increasingly prevalent group of neglected tropical diseases.

Keywords IMMUNOSTIMULATION; MACROPHAGES; HIGH CONTENT SCREENING.

4.4 EPIDEMIOLGY/ECOEPIDEMIOLOGY/MOLECULAR EPIDEMIOLOGY/PREVENTION AND CONTROL

O5-01: RETROSPECTIVE EPIDEMIOLOGICAL ANALYSIS OF VISCERAL LEISHMANIASIS IN COLOMBIA

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Overall, Leishmaniasis in its different clinical forms are neglected tropical diseases associated with poverty and caused by several species of *Leishmania*. Historically, the most severe form is Visceral Leishmaniasis, which is endemic in 56 countries worldwide. For the specific case of South America, the countries with the highest visceral leishmaniasis reports are Brazil, Venezuela, and Colombia. Considering the importance of this disease in terms of public health and the scant data analysis nationwide in the different endemic areas, we proposed to make the first metadata analysis of this disease in Colombia. We collected public data available in the National Public Health Surveillance System (SIVIGILA), National Health Institute (INS), National Administrative Department of Statistics (DANE), and scientific publications related with Visceral Leishmaniasis in Colombia. The analysis was focused on the demographic characteristics, spatial distribution, temporal occurrence of visceral leishmaniasis cases between 2007 to 2018, and the distribution of vectors in the endemic departments. A total of 306 Visceral Leishmaniasis cases were reported to SIVIGILA for the period, with nationwide coverage of 25.5 cases/year, being 2007 and 2012

the years with the highest and lowest number of confirmed cases respectively. The analysis showed that children under 7 years represent the most cases, mainly in the subsidized health regimen, fortunately, just 2.28% related deaths were reported in the period analyzed. Considering the geographic origin of patients, we identified a total of 42 municipalities from 10 departments, where the historical ones were Huila, Sucre, Cordoba, and Bolivar, and new scenarios as La Guajira and Cesar in the last years. When we integrated these findings with the spacial and temporal changes with the vector distribution, we identified that mainly *Lutzomyia longipalpis* and *Lu. evansi* are present in the historical and new departments. With this data overlap, and considering other variables, we proposed hypotheses about the changing panorama of this disease in the country such as natural phenomena, migratory movements, economic activities, and availability and richness of mammalian reservoirs of *Leishmania*. The findings showed in this investigation increase our knowledge and understanding about Visceral leishmaniasis and hold up the importance to prioritize the areas with changes in the disease occurrence to improve the prevention and control programs in Colombia.

Keywords: VISCERAL LEISHMANIASIS; EPIDEMIOLOGY; VECTOR; COLOMBIA

O5-02: EPIDEMIOLOGICAL TRENDS OF CUTANEOUS LEISHMANIASIS IN FRENCH GUIANA: A 5-YEAR RETROSPECTIVE STUDY

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Several epidemiological characteristics of Cutaneous Leishmaniasis (CL) in French Guiana (FG) need clarification. Previous studies have suggested that years with less rainfall would be associated with higher incidence. Higher incidences were reported in the Maroni area and among Brazilian gold miners. Recent data have also hinted to the emergence *Leishmania braziliensis*, as well as pentamidine-resistant strains of *L. guyanensis*. Our aim here was to confirm these trends over the last 5-year period in FG. We retrospectively included all cases of proven CL (compatible lesions and at least one positive test among smear, culture and PCR) seen in the Cayenne Hospital Center between January 1st 2017 and December 31st 2021. In total, we recorded 887 proven cases of CL. The mean yearly incidence was 3.2/10 000 inhabitants, with average years like 2017 and 2018 (160 cases, 5.6/10 000), lower incidences in 2019 (56 cases, 1.9/10 000) and 2021 (94 cases, 3.3/10 000) and a record high incidence in 2020 (413 cases, 14.3/10 000). Two-thirds of patients (594, 67.3%) were men. Their origin was known in 624 cases. Brazilians represented the largest group (319 patients, 51.1%). The area of contamination was identified in 615 cases, mostly along the Maroni river (273 cases, 44.4%), followed by the coast (242, 39.3%), the Oyapock (78, 12.7%) and the central region (22, 3.6%). PCR was positive in only 43.8% of patients in 2017 but its sensitivity increased to 90% after

2018, with the introduction of swab samplings and SYBR Green PCR targeting Hsp70. *L. guyanensis* was by far the most frequent species (559 cases, 63.0%), followed by *L. braziliensis* (85, 9.6%), *L. amazonensis* (13, 1.4%), *L. lainsoni* (9, 1.0%) and *L. naiffi* (4, 0.5%). The predominance of *L. guyanensis* decreased during the dry year of 2019 (29 cases, 61.7%) and increased during the wet year of 2020 (309, 83.7%). Conversely, *L. braziliensis* remained stable (10-20 cases/year) throughout the study period. In total, 25 patients with *L. guyanensis* (25/559, 4.5%) and three with *L. amazonensis* (3/13, 23%) presented a clinical failure with the first-line treatment by pentamidine. Patients infected with *L. braziliensis* were treated with meglumine antimoniate until 2018 when amphotericin B replaced this drug with a similar success rate (75%). These results highlight the very specific dynamics of CL in French Guiana. The persistence of CL infections seems to be mostly driven by Brazilian gold miners working on recently deforested areas. The relation between climate and CL is still incompletely understood in this territory. Previous studies have suggested that EL Niño years would be associated with higher incidence in French Guiana, but the very low incidence observed during 2019, one of the driest ever year sin FG, does not support this link. The combination of a dry season followed by intense rainfalls seems necessary to ensure important sandfly populations and activity. The incidence of *L. braziliensis* remained stable but, combined with pentamidine-resistant *L. guyanensis*, this species represents a therapeutic challenge which calls for the development of new treatment options.

Keywords *Leishmania lainsoni; Leishmania naiffi; Leishmania braziliensis; Leishmania guyanensis*

O5-03: EPIDEMIOLOGY OF A RECENT VISCERAL LEISHMANIASIS OUTBREAK IN EASTERN KENYA

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Visceral leishmaniasis (VL) is a neglected tropical disease caused by a bite with an infected sandfly. It is fatal if left untreated and therefore failure to control the disease through populations and the sandfly measures can lead to recurrent disease outbreaks. The study aimed at determining the prevalence, risk factors and distribution of VL outbreak from May 2021 in Tharaka Nithi County. This is the first time for an outbreak with fatalities to be reported in the area. The study was conducted in affected regions of Tharaka North and South sub counties. The community members were mobilized through assistance of the Public Health Officers to a central data collection point. A cross sectional study was done where data collected included whole blood using dried blood spot (DBS), buffy coat smear and questionnaires on the populations' demographics, risks factors, clinical and health seeking behaviours. These samples and information were transported to the KEMRI laboratories for analysis. DNA was extracted from the DBS and analysed using Polymerase Chain Reaction (PCR), whereas microscopic analysis was performed from buffy coats, stained in geimsa. Prior to data collection, necessary approvals were sought including the participants' informed consent. A total of 720 samples were collected and analysed, out of which, 174 were positive giving a prevalence of 24.4% which was distributed equally among all ages. Out of this, not all exhibited clinical symptoms of VL. 31% had fever, 28% had a rash, and 15% had swelling of lower limbs. Considering other clinical features of VL, 12% of the

cases had increased abdominal volume, bleeding in 6%, and 8% had splenomegaly (enlargement of the spleen). 2% had previously been diagnosed with VL indicating recurrence of the disease. There were populations' risks that contributed to this positivity. 30% lived in a mud walled house with 78% of all cases living in houses with cracks on the walls. Generally, 80.9% of the population sampled live in a mud-walled house with 78.5% having cracks on the wall showing a risk for spread. 15% of the bites in the cases occurred in the evening when outside the house (57%) most of whom (61%) did not use a sandfly control measure such as replants. Other risks identified included not sleeping under a treated mosquito net at 41% despite 53% of them sleeping under a net. The state of the nets was however questionable in relation to the number of bites that occur inside the house which stood at 40%. In conclusion, there is a threat to occurrence of another outbreak in Tharaka Nithi. The reduction of VL cases will require collaborative effort between the different government ministries. This will enable community education on preventive measures and risks at hand. Further, provision of Indoor Residual Spraying (IRS) of the households and other preventive measures such as Insecticide Treated Nets (ITNs) by government and research on the transmission patterns and extend of the disease through continued surveillance, monitoring and evaluation of VL impact is required.

Keywords VISCERAL LEISHMANIASIS; POLYMERASE CHAIN REACTION; QUESTIONNAIRES; OUTBREAK

O5-05: IMPLEMENTATIONSCIENCE OF VISCERAL LEISHMANIASIS (VL) ELIMINATION IN NEPAL

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Nepal with Bangladesh and India, signeda Memorandum of Understanding (MoU) for the visceral leishmaniasis (VL) elimination from the regionin 2005. The VL elimination programme includes the strategies of early case detection and effective treatment, vector control, community participation, and operational research. Since 2005,the Special Programme for Research and Training in Tropical Diseases (WHO/TDR), has been coordinating and financing implementation research as well as conducting clinical trials in support of the VL elimination initiative in the Indian sub-continent. The objective of this study is to conduct an in-depth review on VL research along with the assessment of the contribution and impact of implementation research on the VL burden over the last 15 years. To the highlights of the research findings belong: i)rK39 wasvalidated and used as a confirmatory test for VL since 2005; ii) miltefosine replaced sodium stibogluconate as a first line of treatment in 2008 to 2014. iii) liposomal amphotericin B replaced miltefosine in 2015 as a result of increasedtreatment failures and relapse rates (depicted in TDR funded implementation researchassessingthe pharmacovigilance information of miltefosine); iv) combination therapy was also introduced in Nepal's national protocol of treatment in 2014; v) active case detection with its standard operational procedures (SOPs) was also incorporated into the national protocol of VL elimination vi) integrated vector management including indoor residual spraying, long lasting insecticidal bednets, environmental vector

management, insecticidal wall painting, slow-release insecticides for bednet impregnation applied by local communities can significantly reduce vector densities and contribute to the reduction of VL transmission. Regular meetings of programme managers with researchers sharing research questions and discussing potential solutions have led to evidence based policy changestranslating research findings into programmatic activities.The Regional Technical Advisory Group`s (RTAG) recommendationsonnew strategies also contributed to the successful elimination programme with its target of 1 case /10 000 inhabitants in the district. VL case numbers are now reduced by 90% compared to those in 2005. This successful model of implementation research for VL elimination can be replicated in other countries with diseases targeted for elimination.

Keywords VISCERAL LEISHMANIASIS ELIMINATION; NEPAL; IMPLEMENTATION RESEARCH; STRATEGIES

O11-01: ACTIVE CASE DETECTION AND SANDFLY CONTROL STRATEGIES FOR THE CONSOLIDATION AND MAINTENANCE PHASE OF THE VISCERAL LEISHMANIASIS ELIMINATION IN NEPAL

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Nepal has entered into the consolidation and maintenance phase of visceral leishmaniasis (VL) elimination. In order to protect the achievements made so far, it would be particularly important to detect cases early in order to start treatment and apply vector control measures to avoid the spread of the infection. This study was conducted to identify novel approaches to finding cases and transmission foci early that can be, if successful, incorporated into the national VL elimination programme during the ongoing consolidation and maintenance phases. This was an evaluation study using mixed methods. A baseline study including integrated active case detection (ACD) of febrile patients and determination of sandfly densities in 9 villages was conducted in November 2019. Vector control interventions including Insecticide Residual Spraying (IRS) in 222 households of two villages, Insecticidal Wall Painting (IWP) in 33 households of one village and 698 bed net impregnations (IBN) from 242 households of three villages were performed. The final follow-up of active case detection through household screening and the measurement of vector densities was conducted at 16 months of vector control interventions. VL programme managers at district and community level were interviewed. Community members were interviewed to assess their satisfaction with vector control interventions. At baseline, 2336 people from 416 households were screened detecting 24

febrile cases but none of them were VL positive. Three past VL cases and one past tuberculosis case were identified in baseline screening. Follow up final screening of 2334 individuals from 433 households identified one past VL and one past leprosy case within the last 12 months. There were no febrile cases during follow up screening. IRS was not found to be effective in reducing sandfly densities at 16 months after spraying whereas IWP and IBN were found to be effective in reducing sandfly densities during the same period. More than 90 percent of respondents in the three vector control arms (IRS, IWP and IBN) reported that there was a reduction in sandfly/mosquito density after intervention. Mild side effects were reported by very few individuals. Active case detection of VL is an important activity during the attack phase of the elimination program and may still be important in communities where a VL index case or a surge of febrile illnesses has been detected ("focal ACD"). Insecticidal wall painting and use of impregnated bednets were found to be effective to reduce the sandfly density even upto 16 months after the intervention, measures which could be included in the post-attack phase of the VL elimination program in the Indian sub-continent.

Keywords VISCERAL LEISHMANIASIS; ACTIVE CASE DETECTION; SANDFLY CONTROL; ELIMINATION PROGRAM

O11-02: INCORPORATION OF INSECTICIDE-IMPREGNATED COLLARS: METHODOLOGY USED BY THE BRAZILIAN MINISTRY OF HEALTH TO CONTROL VISCERAL LEISHMANIASIS

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The Visceral Leishmaniasis Surveillance and Control Program (PVC-LV) of the Ministry of Health (MOH) of Brazil is responsible for developing national guidelines for facing the disease. The lack of concrete evidence regarding the effectiveness of strategies proposed for its control, with regard to the reduction of human and canine cases, raised the need to review the program's actions. In order to evaluate and promote new control tools for the program, the MS financed, in 2010, a controlled, multicenter intervention study with the aim of evaluating the effectiveness of insecticide-impregnated collars (4% deltamethrin) in endemic municipalities in Brazil. The result of the study showed that, associated with the other control actions recommended by the program, the use of the collar was responsible for a 50% reduction in the prevalence of the disease in dogs in the intervention areas when compared to the control areas. This work aims to describe the methodology used by Brazil, in the incorporation of collars impregnated with insecticide as a tool to control visceral leishmaniasis (VL). As a definition of the municipalities eligible for incorporation of the strategy, the PVC-LV used a composite index, which includes the average of cases and the coefficient of incidence of human cases in the last three years. Minimum requirements were also defined for the municipalities on structural, operational and technical aspects for carrying out the collaring action. Furthermore, the creation of Local Work Areas (ATL) in the municipalities was recommended. To define these areas, the cumulative incidence coefficient of VL and at least one of the following

indicators were considered: ratio of dog to inhabitants, canine prevalence and/or socioeconomic vulnerability. Then, based on the frequency of case registration and average incidence of VL in the last four years, the ATL were stratified into low, medium or high priority for implementing the intervention with insecticide-impregnated collars. For the purpose of evaluating the collaring of dogs, monitoring of morbidity indicators in humans and dogs, and operational indicators, such as the proportion of dogs collared, was foreseen. Regarding the vectors, the indicators defined were household infestation and relative abundance. In addition, other measures were established to be met by the municipalities, such as, for example, the elaboration of an action plan with strategies for incorporation; maintain the action for at least four years of work in the elected areas; and minimum coverage of 90% of estimated dogs per collar cycle. Finally, considering the operational complexity involved in dog collaring, it is essential to establish a methodology and criteria for the execution and monitoring of the intervention, in order to ensure that its implementation takes place in the most appropriate way possible.

Keywords VISCERAL LEISHMANIASIS; IMPREGNATED COLLARS; RESERVOIR

O11-03: ENTOMOLOGICAL MONITORING OF INSECTICIDE-IMPREGNATED COLLARS TO CONTROL OF VISCERAL LEISHMANIASIS

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In 2021, the Brazilian Ministry of Health announced that insecticide-impregnated collars would be implement throughout the country as a control tool for VL, mostly in municipalities with high, intense and very intense transmission of the disease. To be eligible, in addition to the epidemiological classification, the municipality has to show operational capacity and adequate infrastructure to carry out surveillance and management activities of reservoirs and vectors. For vectors, the recommendation is to do an entomological monitoring before and during the intervention to understand the impact of the tool over time. The objective of this work was to evaluate the frequency of sandflies in areas where collars impregnated with insecticide will be use. Sandflies were captured at 20 points distributed throughout the municipality of Três Lagoas/Mato Grosso do Sul, with 10 points in the control area (areas without future use of collars on dogs) and 10 points in the intervention area (areas with future use of collars on dogs). The captures were carried out using CDC light traps, set three consecutive nights a month, from 6 pm to 6

am, from July/2021 to January/2022. Two traps were placed at each point, one inside and another outside the house, close to the animal shelters. Sandflies were identified using the classification by Galati (2003). Descriptive analysis of household infestation data and relative vector abundance were performed, considering the different areas (control and intervention). During the study period, 3,008 sandflies were captured, 99.9% of the *Lutzomyia longipalpis* species. Of the total, 1,839 (61.14%) sandflies were captured in the control area and 1,169 (38.86%) were captured in the intervention area. Household infestation ranged from 40 to 80% in the control area and 30 to 80% in the intervention area, indicating similarity in the distribution of sandflies in both areas. The average number of sandflies per household (relative abundance) in the control area (173 specimens) was near 50% higher than in the intervention area (89 specimens), indicating a difference in density between the areas. These results indicate differences in the density of sandflies in the control and intervention areas of Três Lagoas/MS. These data are essential for establishing the baseline for the evaluation of vector behavior during the use of insecticide-impregnated collars.

Keywords VISCERAL LEISHMANIASIS; *Lutzomyia longipalpis*; CONTROL; INSECTICIDE-IMPREGNATED COLLARS

O11-04: DELTAMETHRIN-IMPREGNATED DOG COLLARS FOR THE VISCERAL LEISHMANIASIS CONTROL IN BRAZIL: A PROSPECTIVE STUDY TO ASSESS THE IMPACT ON VECTOR PARAMETERS

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The Brazilian visceral leishmaniasis (VL) control program focuses on the early diagnosis and treatment of human cases, infected dogs' euthanasia, and vector control. Recently, deltamethrin (DM) impregnated collars to prevent VL was incorporated in areas with high and intense transmission. Field studies show that protecting dogs with DM impregnated collars contributes to decreasing the VL incidence in humans. The insecticide and repellent effect of collars aim to affect the vector population; however, the impact on vector capacity parameters has not yet been evaluated. Here we describe the study design to assess the impact of the mass use of DM impregnated collars on ecological parameters such as blood-feeding habit, vector abundance, and *Leishmania infantum* circulation in areas where this strategy has been implemented in Brazil. The study included eight Brazilian municipalities classified into high or intense VL transmission according to the PAHO classification. In each municipality, an area with at least 90% of the dogs was selected for delivering DM impregnated collars (intervention), and other areas with similar environmental characteristics, human development index, and canine prevalence were selected as control. In each area, ten blocks spatially distributed randomly were selected, and a house in each block favorable to sandflies capture was included. In each domicile, two light traps were installed monthly for three consecutive days (40 traps in each municipality). The sandflies capture began in August 2021 and will

be performed monthly for 24 months, during three successive nights. Additionally, a questionary about environmental variables, the number of domestic animals (blood sources), and the loss of dog collars (in the intervention area) is applied monthly. Those variables will be used in the analysis to control potential bias in the estimation of vector density and blood feed index. Among the 77 and 80 domiciles in the control and intervention areas, respectively, 95% have dogs, and 62% have chicken houses. Sandflies captures began in August 2021, and to March 2022, 1.280 domiciliary visits were performed, with 3.840 capture attempts and 692 positive samples. In two months (August and September), 10.296 *Lutzomyia longipalpis* specimens were captured (2278 females and 8018 males). In the control and the intervention areas, 1043 females and 3973 males, and 1234 females and 4041 were captured, respectively. Results from the follow-up of this study on vector abundance and markers of vectorial capacity will provide information for managers of leishmaniasis control programs that might improve the delivery and monitoring of the DM collar in priority areas for VL control.

Keywords VISCERAL LEISHMANIASIS; CONTROL; SANDFLIES; DELTAMETHRIN; COLLARS; CANINE

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O11-05: FIELD EVALUATION OF MOSQUITO NETS IMPREGNATED WITH LONG-LASTING INSECTICIDE (LLIN) FOR SAND FLIES IN BRAZIL

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Among the vector control measures, the use of mosquito nets impregnated with long-lasting insecticide (LLINs) is widely used for *Anopheles* spp control in endemic areas of malaria. There are few studies that evaluate the use of LLINs as a prevention and/or control measure for the sand flies, vectors of leishmaniasis. The objective of this study was to evaluate, in the field, the repellent/insecticide effect of mosquito nets impregnated with insecticides. For this purpose, the experiments were carried out in three rural areas of Ceará state in Brazil in September and November/2021and January/2022 using CDC light traps baited with the compound hexanol. The light traps were covered with mosquito nets, impregnated or not with insecticide and were left in the environment from 18:00h to 06:00h. The design of the experiments were a 4X4 Latin square with the following treatments: a) mesh impregnated with alphacypermethrin (Interceptor®); b) mesh impregnated with alphacypermethrin and chlorfenapyr (Interceptor G2®); c) mesh not impregnated with insecticide; d) control, the light trap without any cover. In the last capture, January/2022, the bulb of light was removed from the CDC trap and only hexanol was used as the attractant. A total of 41,972 sand flies were collected during the experiments. As expected, in the experiment without light, only with hexanol, the amount of sand flies was much lower. *Nyssomyia whitmani* was the most abundant species collected in September/2021 and *Migonemyia*

migonei in November/2021 and January/2022. The Interceptor G2 screen was the only mesh in which sand flies were captured in smaller numbers than the control in November/21 and January/22.

Keywords Sand flies, control, insecticide-impregnated mosquito nets, Interceptor

O12-01: INCREASING INCIDENCE OF VISCERAL LEISHMANIASIS RELAPSE IN SOUTH SUDAN, 2001-2018

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Visceral Leishmaniasis (VL) is a fatal and neglected vector borne disease, which is endemic in South Sudan, where it causes high yearly seasonal caseloads and periodic major outbreaks. MSF has provided health care in South Sudan since the late 1980's, including treatment for 67,000 VL patients. In recent years, MSF's monitoring data showed increasing numbers of VL relapse cases. These patients need retreatment to survive, while access to treatment is poor. Moreover, a decreasing efficacy of the currently used VL treatment regimen is problematic as there are no alternatives, although a new regimen is in development. A retrospective analysis of routine data was performed in order to analyse trends and provide insight into the possible causes of this increase. Programme data from MSF's hospital in Lankien, Jonglei State, South Sudan, for the period 2001-2018 were analysed to detect trends in VL relapse cases using Joinpoint regression. Routinely collected patient-level data from the same period were analysed to describe patient characteristics and treatments received. VL relapse as a proportion of all VL cases increased by 6.5% per annum (95% CI 0.3% to 13.0%, p=0.04), from 5.2% during 2001-2003 to 14.4% during 2016-2018. Primary VL and VL relapse patients had similar age, sex and anthropometric characteristics, the latter indicating high indices of undernutrition, which were relatively constant over time. Clinical factors (Hb, spleen size, and VL severity score) also did not vary substantially over time. Sodium stibogluconate and paromomycin combination was the main treatment

regimen from 2001-2018, used in 68.7% of primary and 70.9% of relapse VL cases; AmBisome was introduced in 2013, received by 22.5% of primary VL and 32.6% of VL relapse cases from 2013-2018. The increasing incidence of VL relapse does not appear to be explained by changes in patient characteristics or other factors. Therefore, the effectiveness of treatment regimens warrants further investigation as a causal factor. The main limitation of our study is that patient-level data were subject to error, and there were missing data, particularly in years when patient-level data collection was interrupted due to insecurity. Given these limitations, MSF is planning a prospective observational study to assess the incidence of relapse after treatment for primary VL in South Sudan in which patients will be followed up for 12 months. The results of this prospective study will indicate whether drug sensitivity monitoring of anti-leishmanial drugs in East Africa is warranted. This underscores the pressing need for new chemical entities that will enable safe and highly effective short-course oral treatments for
VL.

Keywords VISCERAL LEISHMANIASIS; RELAPSE; SOUTH SUDAN; SODIUM STIBOGLUCONATE; PAROMOMYCIN

O12-02: CHARACTERISTICS OF IMPORTED CUTANEOUS LEISHMANIASIS IN HAMBURG, GERMANY FROM 2015 TO 2021

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In Germany cutaneous leishmaniasis is an imported disease. Migrants, tourists, workers and visiting friends and relatives are at risk when travelling to endemics countries. We analysed data of all patients with cutaneous leishmaniasis presenting at out outpatient department for tropical medicine and tropical dermatology in the years 2015-2021. In total, 104 cases of imported cutaneous leishmaniasis were seen in this period. *Leishmania infantum* was the predominant species (41%). *L. major/L.tropica* accounted for 33% of the total number of cases. 63% of patients were male and the majority were tourists (56%). Children accounted for 26% of the total cases. The most frequent origin of infection was Mallorca, Spain (34%), followed by Syria (18%). Two patients had mucosal involvement by continuity. First-line treatment was a systemic therapy in the majority of cases (24% liposomal amphotericin B, 18% miltefosine). 30% of patients received intralesional therapy with meglumine antimoniate, whereas in 16% of the patients a "wait and see" strategy was used. In Germany cutaneous leishmaniasis is not notifiable. In our outpatient department, a referral center in tropical medicine in Northern Germany, we observed a high number of imported cutaneous leishmaniasis in travellers and migrants. Although, many cutaneous leishmaniasis of the old world are self-healing, a systemic therapy is needed in progressive stages. In non-endemic countries cutaneous leishmaniasis

surveillance is necessary to improve the diagnosis and therapeutic options for people at risk.

Keywords CUTANEOUS LEISHMANIASIS; IMPORTED; TRAVEL; SURVEILLANCE; HAMBURG

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O12-03: EVOLUTION AND FACTORS ASSOCIATED WITH THE OCCURRENCE OF VISCERAL LEISHMANIASIS IN THE STATE OF SÃO PAULO, BRAZIL

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Bioecological, social and individual factors determine visceral leishmaniasis (VL) occurrence. However, the role of environmental changes resulting from the expansion of roads/railways and agriculture has been little studied, especially sugarcane production. Sugarcane production grew continuously in Brazil leading to the reorganization of the geographic space, and modifications of the landscape and the sociodemographic profile of the regions. Ecological study describing factors associated with human VL in the state of São Paulo (SP), Brazil, from its introduction in 1999 to 2014. Two analytical approaches were used: (Analysis 1) including all municipalities of SP, with and without human VL cases, and (Analysis 2) including only the cities with VL cases; in which the outcome was the occurrence of cases in each year. The exposure variables were of two types: socio-environmental (altitude, highway crossing the city, presence of a railway in the municipality, presence of a sugarcane mill, gross domestic product (GDP) per capita, area/ha with sugarcane, and the presence of planted area) and climate (minimum, average and minimum annual temperatures; evapotranspiration and average annual precipitation; monthly average of rainy days; considering the period 1997-2014). In “analysis 1”, the logistic regression model was used. In “Analysis 2”, multilevel logistic regression was used, which considers repeated measures of the occurrence of VL cases

over the years in each municipality. Associations were expressed as odds ratios and 95% confidence intervals. Analysis 1: there was a reduction in the chance of the municipality has registered a case of VL between 1999-2014 of 34% for every 100 meters of increase in altitude and 5% for each increase of R$1000 in GDP per capita. The municipalities with a railway and with sugarcane plantations had 24 and 2.6 greater chances of recording VL cases, respectively. The presence of a highway and a sugar cane plant were not associated with VL. In the adjusted model, we observed: 1) an increase of one degree in maximum, minimum, and average temperatures was associated with a 2.3 to 3.3 times increased chance of VL; 2) for each millimeter of increase in evapotranspiration and precipitation, the chance of VL increased by 16% and 14%, respectively; 3) with each additional day of rain, the chance VL occurrence decreased ~50%. Analysis 2: After adjustment we observed 1) for each millimeter of increase in precipitation, the chance of VL occurrence was reduced by 2%; 2) for each additional day of rain, the chance of VL increased by 21%; 3) evapotranspiration rate and temperature were not associated with VL. Climatic factors directly influence the occurrence of VL transmitted by *Lutzomyia longipalpis*, a typical vector of warmer regions with low relative humidity. The expansion of the disease was associated with environmental changes in western SP, promoted by an increase in sugarcane cultivation and the impoverishment of areas where railroads were deactivated. Local surveillance and control programs will need to mobilize resources and employ multisectoral approaches, integrating the social and structural features of the municipality, including the physical environment, social conditions, and climate change.

Keywords VISCERAL LEISHMANIASIS; ECOLOGICAL STUDY; FACTOR ASSOCIATED; MULTILEVEL LOGISTIC REGRESSION

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O12-05: LEISHMANIASES IN BOLIVIA: EPIDEMIOLOGICAL UPDATE

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We present a comprehensive review and unpublished results about leishmaniases in Bolivia, that is endemic in cutaneous (CL) and visceral leishmaniases (VL), with around 2,000 annual cases. In 2020, 2,059 cases were reported (1,861 CL = 90.4%, 198 ML = 9.6%), a high incidence with 28.3 cases per 100,000 population. Authorities report CL in seven of the nine departments; nevertheless, the local transmission was not proved in Chuquisaca. Leishmania braziliensis is the more widespread agent of CL and mucosal leishmaniasis (ML). The transmission of L. braziliensis is sylvatic in the lowlands, involving to Psychodopygus yucumensis, Ps. llanosmartinsi and Ps. carrerai carrerai. While, in the sub-Andean ancient settlements, is predominantly domestic and peridomestic by Lutzomyia nuneztovari anglesi. The progressive transition to domestic transmission, affects more people of different age and sex, frequently with several lesions, some in the face. Leishmania amazonensis is the second species involved in CL, with sylvatic transmission by unknown vector(s) in Santa Cruz and Cochabamba, and predominantly domestic in Sub-Andean region of La Paz (1000-2000m) by Lu. nuneztovari anglesi, the vector in sympatry of both L. amazonensis and L. braziliensis. Recently, we identified a case of cutaneous diffuse leishmaniasis in Pando, and the first cases in a cat and a monkey from the border between La Paz, and Beni Departments; according our preliminary results the species involved seems to be L. amazonensis (first cases). While, L. lainsoni and L. guyanensis, were occasionally the agents of CL in La Paz and Cochabamba. During the last 20 years, we identify an increment of co-endemicity due to L. braziliensis and L. amazonensis in different foci. As conclusion, CL due to L. braziliensis and L. amazonensis occur in a variable

range of transmission between predominantly domestic and sylvatic patterns in different foci, with increasing progressively the importance of L. amazonensis as human pathogen wide spreading. In 2021, in the Chapare (Cochabamba), were identified in patients with CL and ML by sequencing, L. braziliensis (59%) and a particular sub-population named L. braziliensis outlier (39%), and surprisingly, one autochthonous case of ML by L. peruviana. In relation to VL, sporadic cases were reported between 1983-2020; one in 2019, two in 2020, and recently (2022), a new case in the southern Chaco region of Tarija (the first one). In contrast a recent publication declares "Seventy nine VL cases have been reported spanning 36 years (1982 - 2018). Fifty six of these had Visceral Leishmania, and 23 had Leishmania sp. Infections." From them 35 cases were reported between 2012-2018, with 31 (89%) without clinical data. Thus, these data are completely refutable. Lutzomyia longipalpis is the proven sub-Andean vector of *L. infantum*. The other potential or suspected vectors are Nyssomyia shawi for L. braziliensis and L. guyanensis in Cochabamba. Nyssomyia neivai for L. braziliensis in Tarija and Trichophoromyia velascoi for L. lainsoni in La Paz. The control is limited to passive detection and treatment of cases with pentavalent antimonials and amphotericin B. Our studies demonstrated the potential efficacy of insecticide-impregnated curtains in reducing domestic transmission.

Keywords Leishmania; LEISHMANIASIS; BOLIVIA; SANDFLIES

O19-01: PREDICTING VISCERAL LEISHMANIASIS IN HIV INFECTED PATIENTS IN ETHIOPIA: FIRST STEP TOWARDS A SCREEN AND TREAT STRATEGY

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HIV coinfection is one of the key challenges for control and management of visceral leishmaniasis (VL). VL-HIV coinfection rates are particularly high in NW-Ethiopia, reaching 20-40% of all VL cases. Once *Leishmania donovani* infection has evolved to the disease stage VL, prognosis at the individual level is dire, with many patients experiencing frequent relapses. Tackling *Leishmania* infection before disease onset would thus be a logical approach. We hypothesized that the period of asymptomatic *Leishmania* infection constitutes a window of opportunity for screening strategies, to capture those at high risk of developing VL. To build the evidence-base for such a strategy, we conducted a prospective cohort study including HIV-positive adults enrolled in HIV care in a VL endemic region in North-Ethiopia. Patients were monitored for *Leishmania* infection and VL development up to two years with clinical and laboratory evaluations every three to six months. Laboratory evaluations included rK39 RDT, rK39 ELISA, DAT, KAtex, *Leishmania* kDNA PCR, CD4 count and HIV viral load. Prevalent *Leishmania* infection was defined as positivity on any of the *Leishmania*

markers at baseline, incident infection as positivity on any marker during follow-up in those with negative markers at baseline. Risk factors for VL were identified using Cox regression. The study was conducted between October 2017 and October 2021. The interim analysis on 396 patients that reached one year of follow-up included 261 (66%) male HIV patients, 56% were between 18-39 years old and almost all were on ART (99%). The median CD4 count at enrolment was 414 cells/µL (IQR 269-599) and 103 (26%) had a history of VL. Prevalent *Leishmania* infection was present in 45%, including positivity on DAT (23%), rK39 RDT (30%), PCR (6%) and KAtex (8%). Over the first year follow-up, 16 developed VL at a median of 150 days (IQR 101-246) after enrolment. Besides a lower CD4 count, male sex, pre-ART and a history of VL, a prevalent asymptomatic *Leishmania* infection was found predictive of VL (odds ratio (OR) 15.4; 95% confidence interval (CI) 2-118), ranging from an OR of 11 (95% CI 3-51) for a positive serological test, to 23 (95% CI 8-70) for KAtex and 62 (95% CI 19-208) for PCR. An incident asymptomatic infection also increased the risk of VL, ranging from an OR of 14 (95% CI 2-105) for an incident positive rK39 RDT result, 28 (95% CI 6-128) for KAtex and 181 (95% CI 19-1711) for PCR. Since the interim analysis, a total of 571 individuals have been recruited and followed up to two years of which 34 (6%) have developed VL. Data cleaning is currently ongoing and statistical analysis is planned to start February 2022. Final results will be available at the time of WL7. We will present a prognostic tool to allow for individual prediction of VL risk at each point during clinical follow-up. After defining which HIV patients are at highest risk of developing VL, these patients can be targeted for prophylactic treatment in a follow-up clinical trial.

Keywords VISCERAL LEISHMANIASIS; HIV; PREDICTION; ASYMPTOMATIC

O19-02: PREVALENCE AND RISK FACTORS OF CUTANEOUS LEISHMANIASIS (CL) IN A SOUTH-ETHIOPIAN VILLAGE NEWLY IDENTIFIED TO BE CL ENDEMIC

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Although there are several areas in southern Ethiopia with environmental features favorable for cutaneous leishmaniasis (CL), studies on the existence and risk factors of CL are lacking beyond a few well-known hotspots. We conducted a survey between July and August 2021 in Bilala Shaye, a village in the Ethiopian highlands at an altitude of 2,250 meters. All community members were interviewed and those with skin lesions were clinically assessed. In the sub-group of individuals with clinical signs of CL, tape disc samples were collected for PCR analysis to confirm CL. Data on individual risk behavior and features of the household and surroundings were collected using standardized questionnaires. Multivariate logistic regression was used to identify independent risk factors of CL (active or scar). A total of 1012 individuals from 252 households were included. The median age was 23 years (IQR 12-50), with 67 participants (7%) below the age of five; 51% were female. All households owned domestic animals, and 143 (57%) had goats/sheep in or around the house. Animal dung was found in the compound of 77% of the households. For 58% of the households, the house was reportedly located within 300 meters of areas where hyraxes live. Availability of bed nets was uncommon (<1%). We identified 25 active suspected CL cases (2.5%), with a median age of 10 years (IQR:6-37, range:1-86). The prevalence reached 7.6% (12/157) in children between 5-12 years old. The median lesion duration at the time of the study was 1.8 months (IQR:0.9-2.7). PCR was positive for 8 out of 9 suspected CL cases from whom a tape sample was collected. Additionally, 382 (38%) participants had a scar due to CL, with a median age of 32 years (IQR:16-45).

Active lesions were predominantly (68%) located on the face (forehead, cheeks, ears, and chin). In multivariate analysis, spending time outside the home in the evening at places where hyraxes reside (adjusted odds ratio (AOR 1.7 (95% CI 1.3-2.3), P-value: <0.001), the presence of animal dung on the compound (AOR 1.7; (95% CI: 1.1-2.5), P-value 0.025), keeping goats/sheep in the house (AOR 1.5; (95% CI: 1.0-2.1), P-value 0.039) and the presence of a stone fence around the compound (AOR 1.6; (95% CI: 1.0-2.4), P-value 0.021) were associated with increased risk of CL (active or scar). Late evening risk activities included fetching water/firewood, farming, and herding animals. There was no association with household features including the construction of the roof, wall, and floor. The high active CL prevalence amongst young children and CL scars increasing with age indicates active and long-standing endemicity. CL is probably much more widely spread in Ethiopia, calling for surveys in high-risk areas across the country. Risk factors included peri-domestic factors as well as factors associated with spending time in the evening close to where hyraxes reside. Further research is needed to assess to what extent there is peri-domestic transmission and the role of animal dung and goats/sheep in disease ecology and transmission.

Keywords CUTANEOUS LEISHMANIASIS; EPIDEMIOLOGY; RISK FACTORS; PREVALENCE; ETHIOPIA

O19-03: SEXUAL TRANSMISSION OF *Leishmania* PARASITES: SHOULD WE BE CONCERNED?

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Parasites of the *Leishmania donovani* complex that cause visceral leishmaniasis (VL) are predominantly transmitted by phlebotomine sand flies, yet other transmission modes including blood transfusion, organ transplantation and congenital transmission have been documented as well. Although demonstrated for several pathogens (*e.g.*, Zika virus, Ebola virus and *Trypanosoma cruzi*), sexual transmission of *Leishmania* has barely received attention. We performed a review on the available literature of sexual *Leishmania* transmission. Briefly, high viable parasite loads and increased inflammatory markers were found in semen of symptomatic and asymptomatic dogs. Moreover, male-to-female sexual transmission in dogs was reported in both non-endemic and endemic countries. While compelling evidence is available for sexual transmission in dogs, information on *Leishmania* transmission through intercourse among humans is limited to two case reports. A female patient displayed vaginal lesions due to *Leishmania* while only her partner had been in a VL endemic country; and a man with lymphoblastic leukemia and VL showed testicular involvement of *Leishmania* parasites. A better understanding on the importance of sexual transmission of *Leishmania* is crucial for proper patient counseling and disease control, but also to assess the potential implications in fertility. Studying sexual transmission of VL in HIV coinfected individuals seems particularly relevant, as tissue and blood parasite loads are higher and wider spread of the parasites throughout their body causes atypical clinical presentations. Therefore, we are performing a pilot study to gather

preliminary evidence on the presence and persistence of *Leishmania* parasites in the semen of male VL-HIV co-infected patients and the underlying immunopathology. A multicentric cohort study is conducted from March to December 2022 in two hospitals in Caruaru and Recife, northeastern Brazil. We are recruiting 20 adult, male VL-HIV coinfected patients and 20 controls that have no history of VL. Patients are checked for genital lesions of which a swab sample is taken, if present. Semen and blood samples are collected from controls and patients as soon as feasible after VL diagnosis and at the end of treatment. All samples are tested for the presence of (viable) *Leishmania* parasites using RT-qPCR, which will indicate whether *Leishmania* parasites can cross the blood-testis barrier. Semen samples are also tested for inflammatory markers (cytokines and chemokines), indicators of oxidative stress and single cell RNAseq immunological profiles associated with a *Leishmania* infection in the semen. This study is the first to investigate the potential of *Leishmania* transmission through sexual intercourse. Findings will incite future research on the epidemiological importance of sexual transmission and potential implications on fertility. At the time of the conference, the literature review and preliminary data of this pilot study will be presented.

Keywords VISCERAL LEISHMANIASIS; SEXUAL TRANSMISSION; SEMEN; HIV CO-INFECTION

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O19-04: AN ENDOSYMBIOTIC VIRUS UNCOVERS THE RECENT EVOLUTION OF A PROTOZOAN PARASITE

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Leishmania braziliensis is a vector-borne zoonotic parasite inflicting (muco) cutaneous leishmaniasis (CL) in Central and South America. The parasite is frequently associated with the double-stranded RNA virus *Leishmaniavirus* (LRV) 1, forming a unique 'matryoshka' infection in mammals. The presence of LRV1 results in an increased risk of treatment failure and mucosal disease in human infections. Phylogenetic studies showed that LRV was most likely present in the common ancestor of *Leishmania*, prior to the divergence of these parasites into different species around the world, and suggest a long-term co-evolutionary history of the *Leishmania*-LRV symbiosis. However, no study has ever investigated their population (co-) structure and diversity on ecological timescales. Here, we aim to obtain insights into the epidemiology of CL in Peru and Bolivia through a joint evolutionary analysis of *L. braziliensis* and LRV1. Our analyses included 80 *L. braziliensis* isolates, 32 of which were positive for LRV1. *Leishmania* promastigotes were cultured *in vitro* and subjected to whole genome sequencing. Full-length genomes of

LRV1 were recovered through transcriptome sequencing of total RNA extractions of the parasites. Population genomic analyses using 425,197 nuclear SNPs revealed that *L. braziliensis* is geographically structured. One *L. braziliensis* lineage is restricted to the Yungas ecoregion in Paucartambo (Peru), while the other two lineages circulate predominantly within the Amazonian rainforests of Bolivia and Central Peru. Levels of linkage disequilibrium were low and distributions of per-site inbreeding coefficients per population were centered around zero, confirming the presence of relatively high within-lineage recombination rates in *L. braziliensis.* A fourth group of parasites is found within both the Yungas and Amazonian rainforests of Peru, and showed signatures of mixed ancestry from all three *L. braziliensis* lineages. We identify nine divergent LRV1 lineages in our set of *L. braziliensis* isolates. The majority of these lineages show a restricted distribution in the Amazonian rainforests (5 lineages) or the Yungas (2 lineages), and their spatial distribution reflects the geographical population structure of *L. braziliensis*. For instance, the two Yungas lineages co-circulate within the same *L. braziliensis* population from that region and are not found elsewhere. One LRV1 lineage from the Amazonian rainforests of Peru is also found in the rainforests of Bolivia, and is probably the result of recent gene flow from Peru to Bolivia. Finally, we identify one LRV1 lineage with a widespread distribution across the Yungas of Peru and that is associated with a group of hybrid *L. braziliensis* parasites. A phylogenetic tree rooted with LRV1 from *L. guyanensis* shows that this Yungas lineage has emerged relatively recently from its ancestors in the Amazonian rainforests. Our results show that – while the main *L. braziliensis* populations and most LRV1 lineages circulate within isolated pockets of suitable habitat – frequent secondary contacts resulted in the widespread distribution of hybrid parasites and their associated LRV1 lineage. Given the implications of LRV1 in clinical outcome, the successful spreading of LRV1 lineages due to hybridization in the *Leishmania* host population may have important consequences towards the epidemiology of CL in the region.

Keywords *Leishmania braziliensis;* LRV1; CUTANEOUS LEISHMANIASIS; POPULATION GENOMICS; LANDSCAPE GENOMICS

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O19-05: PREVALENCE AND DISTRIBUTION OF *LEISHMANIA* RNA VIRUS 1 IN *Leishmania* (*Viannia*) PARASITES FROM SOUTH AMERICA

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Leishmania RNA Virus 1 (LRV1) is found in different species of *Leishmania (Viannia)*, parasites causing tegumentary leishmaniasis(TL) in South America (SA). It is known that the presence of LRV1 in some *Leishmania* strains exacerbates disease severity in animal models and humans, and might be correlated with treatment failure in humans. In Brazil, at least 6 *L.* (*Viannia*) species are associated with TL, and LRV1 has been described in 5 of them. The aim of this study was to determine the prevalence of LRV1 in *L. (Viannia)* strains available at the *Leishmania* Collection from FIOCRUZ (CLIOC) among different species and strains circulating in several Brazilian regions and other SA' Countries. A total of 126 *L. (Viannia)* spp were thawed from CLIOC and cultured in Schneider's media (116 from Brazil and 10 from other SA Countries). Parasites were collected by centrifugation and RNA extraction was performed by the Trizol™ (Invitrogen) method. Extracted RNA was used for cDNA synthesis using SuperScript IV Reverse Transcriptase kit™ (Invitrogen). LRV1 detection was performed by PCR of cDNA yielding a 240-bp product (Primers: LRV F - 5´-ATGCCTAAGAGTTTGGATTCG- 3' and LRV R - 5´-ACAACCAGACGATTGCTGTG – 3'). The amplified fragments (240pb) were visualized on a 2% Agarose gel stained with GelRed™ (Biotium, Hayward, CA, USA). All Brazilian LRV1 positive strains were from states of Amazon

region (Amazonas, Rondônia, and Pará). Only one non-Brazilian strain was LRV1+, a *L. guyanensis* from Andean region of Venezuela. The presence of LRV1 was detected in 49 out 126 strains (38,9%) distributed among different species: positivity among *L. guyanensis* was 66% (26/39), *L. naiffi* 52% (10/19), (2/3) *L. shawi*, and (1/3) *L. lainsoni* were LRV1+. For *L. braziliensis* strains analyzed, 17% (10/56) were LRV1+, but pondering the sampling from the Amazonian region, more than 45% were LRV1+. *L. braziliensis* is currently the only *L. (Viannia)* appointed species causing human disease in Brazilian regions out of the Amazon area and all strains from these other regions were LRV1 negative. No LRV1 was detected in *L. lindenbergi* (n= 3) and *L. panamensis* (N= 4) strains. This study represents the biggest screening of LRV1 in *L.* (*Viannia*) species conducted so far and more strains are still being analyzed. Our findings are consistent with previous surveys showing absence of LRV1 outside the Amazon region, and corroborate the finding that LRV1 is quite frequent among the different species and strains circulating in northern South America. LRV1 surveys are relevant since the presence of the virus is one of the determinant elements for the outcome of infection pathology, potentially associated with the severe forms of the disease. From the present results we will further 1) explore the co-evolution of LRV1 and *Leishmania* (*Viannia*) parasites; 2) select strains naturally positive and negative for the virus to investigate the impact of the endosymbiont on the biology of the parasite and on the course of infection *in vivo* and *in vitro* by *L. (Viannia)* species.

Keywords CUTANEOUS LEISHMANIAIS*; Leishmania (viannia); Leishmania* RNA VIRUS; LRV1

O19-06: LEISHMANIAVIRUS TYPE 1 IS A RISK FACTOR FOR MUCOSAL LEISHMANIASIS OCCURRENCE

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Mucosal leishmaniasis (ML) is a serious clinical form of leishmaniasis that is characterized by destruction of the nasal and/or the oral mucosae and due to unknown reasons appears as a late complication in 5%–10% of cutaneous leishmaniasis (CL) cases produced by species belonging to *Leishmania Viannia* subgenus. Experimental data suggests that strains of *Leishmania* spp. carrying an RNA virus known as Leishmania RNA virus type 1(LRV1) triggers an immunological response that involves the endosomal Toll-like receptor 3 (TLR3) and has been associated with persistence and dissemination of *Leishmania* (V.) *guyanensis*. Moreover, TLR3 gene displays several single nucleotide polymorphisms (SNPs) associated with resistance or susceptibility to viral infectious diseases. The present work evaluated LRV1 and TLR3 gene polymorphisms as risk factors for the occurrence of ML throughout a retrospective case-control study involving 102 patients. Cases were defined as patients with ML (n=33) and controls corresponded to patients who had CL without mucosal lesions (n=69). A subgroup of controls (n=19) was followed up for a median time of 16 years to rule out ML occurrence. Clinical data were recorded from the patients' medical records and cryopreserved biopsies were used for *Leishmania* species identification, LRV type-1 (LRV1) detection and TLR3 (exons 2, 3, and 4) genotypification. Bivariate and logistic regression

analyses were applied to estimate the risk factors associated with ML occurrence. The predominant *Leishmania* species in both groups was *L. (V.) braziliensis.* Multivariate logistic regression indicated that the infection with *Leishmania* spp. carrying LRV1 is a factor linked with the occurrence of ML [OR,8.81, 95%CI 1.72–45.76 and p = 0.009]. Four SNPs on TLR3 gene were identified and showed no association to ML development. Therefore, LRV1 presence is an independent risk factor for developing ML.

Keywords LEISHMANIASIS; MUCOCUTANEOUS LEISHMANIASIS; LEISHMANIAVIRUS; LEISHMANIA RNA VIRUS 1

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O24-01: DIVERSIFICATION PROCESSES AND CONSEQUENCES FOR THE DYNAMICS OF *Leishmania infantum* INFECTION IN THE AMERICAS

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The relatively recent arrival of *Leishmania infantum* in the Americas during European colonization is expected to reduce the diversity of the parasite founding population. Indeed, until recently the New World (NW) parasite population has been considered extremely homogeneous. Conversely, we have exposed a complex neotropical *L. infantum* structure by the phylogenomic, bioinformatics and molecular analyses of 126 *L. infantum* genomes (19 from the Old World, 107 from the NW). We portrayed the geographic dispersion of a recently described, multi-kilobase deletion on chromosome 31 (chr31), which was associated with resistance to miltefosine. There is a widespread distribution and higher frequency of deletion caring (DEL) strains (126 of 177) and the occurrence of a divergent non-deletion (NonDEL) group in the southwestern Brazil. Importantly, until now, this deletion has been detected only among NW strains and represent an ancestral, inherited trait, suggesting that this important genomic change

may be under positive selection in Brazil and thus probably highly relevant to the local dynamics of *L. infantum* infection. Significantly, the deletion spans across the four copies of tetrasomic chr31, the only stable chromosomal amplification within the mosaic aneuploidy of Leishmania parasites. We obtained important results for one of the deleted genes, the ecto-3'-nucleotidase. There is a significant reduction in ecto-3'-nucleotidase activity in DEL compared to heterozygous (HTZ) and NonDEL isolates. The reported importance of this enzyme as a potential virulence factor led us to further determine the biological consequences of different ecto-3'-nucleotidase activity levels. We assessed DEL and NonDEL strains (N=06) from different regions for survival in the presence of neutrophil NETs and interaction with macrophages. Results revealed that promastigote DEL strains survived less after the interaction with NETs and infected less macrophages after 24 hours. The reduced ability of DEL strains to survive NETs is possibly linked to the reported role 3'ecto-nucleotidase has in the escape of the parasite from this DNA-based traps. Additional in vivo-based experiments will clarify whether such difference in virulence remains. Our work further uncovered six samples that had an intermediate read-depth profile within the chr31 deletion site. These strains were confirmed as caring a heterozygous deletion, resulting from the hybridization between DEL and NonDEL and exhibiting the enzymatic activity restored. The deletion includes four open reading frames displaying an essential nature in parasite viability and infectivity, and even though, the DEL genotype is widely spread and in higher frequency in Brazil. Together, these results highlight the pivotal roles and interplay of genetic exchange vs demographic history in shaping *L. infantum* sequence, karyotypic diversity and biological/phenotypic traits in the New World. Our next goal is to further investigate which compensatory mechanisms have evolved in Brazilian *L. infantum* strains that allow for their deletion to occur and expand in Brazil after its introduction by the Hispanic conquest.

Keywords *Leishmania infantum*; BRAZIL; VISCERAL LEISHMANIASIS

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O24-02: THE IMPACT OF DRUG RESISTANCE OF VISCERAL *Leishmania* SPECIES ON THE PARASITE- VECTOR-HOST INTERACTION

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At the moment only few chemotherapeutics are approved for the treatment of visceral leishmaniasis and these are confronted with increasing treatment failure rates and the emergence of drug resistance. We aimed to predict the potential effects of miltefosine (MIL) and paromomycin (PMM) resistance on the parasite life cycle in the mammalian host and sand fly vector. To evaluate the impact of drug resistance, MIL and PMM resistant *Leishmania donovani* and *L. infantum* strains were experimentally selected *in vitro.* The resulting parasites were phenotypically and genotypically characterized in comparison to the original wild-type population. Moreover, their adaptive behaviour in different sand fly species was studied in order to predict the effects on parasite transmission. Mutations in the MIL transporter (MT) gene are sufficient for acquisition of MIL resistance and are linked to a clear reduction of parasite fitness in mice and sand flies. PMM resistance seems multifactorial with genetic changes predominantly in genes involved in transcription, translation and protein turn-over. PMM resistant parasites develop normally in the insect vector and higher parasite burdens in the mammalian host suggest efficient transmission of this resistance trait. The

drug-dependent changes of parasite fitness indicate that not all drugs are at risk of an immediate spread of resistance. Nevertheless, vigilant drug use and research on resistance-associated phenotypic traits throughout the parasite life cycle continue to be important to safeguard current and future drugs.

Keywords *Leishmania*; RESISTANCE; TRANSMISSION

O24-03: XENODIAGNOSIS TO EVALUATE TRANSMISSIBILITY OF VISCERAL LEISHMANIASIS - HIV COINFECTED PATIENTS LIVING IN AN ENDEMIC AREA OF BIHAR, INDIA

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Despite considerable government effort, visceral leishmaniasis (VL) is still a major public health challenge in India caused by protozoan parasite *Leishmania donovani* and transmitted by the bites of blood sucking vector sand fly *Phlebotomous argentipes*. The spread and severity of infection is exacerbated by its status as an important co-infection of AIDS patients and the overlap in prevalence of HIV and leishmania species. We therefore performed xenodiagnosis to evaluate infectiousness of VL-HIV patients to sand flies in an area endemic for visceral leishmaniasis. A total 732 sand flies were exposed on 14 VL-HIV patients (including forearm and leg; Age: 45.35±11.7, Sex (M/F): 9:5) with a mean of 60 flies per patient (30 flies in each feeding chamber). We observed that only 16.66 % (122/732) of blood fed flies were found positive by microscopy. These patients have significantly higher blood parasitemia compared to VL and PKDL as quantified by qPCR and the median parasitemia was roughly 42205 genomes / ml blood. Importantly, 92.8 (13 out of 14) HIV-VL patients transmitted infection to flies as reveled by qPCR and or microscopy. We then modelled the proportion of flies that get infected based on blood parasitaemia, dividing the VL-HIV patients into three groups defined by

qPCR values. We found that the probability of infection in any given blood fed fly correlated with severity of the disease (as defined by blood parasitemia), ranging from 4.92 % (95% CI 1.31 -16.39) in group 1 patients qPCR (0, 1e+04). to 15.39% (95% CI 4.11 – 43.55) in group 3 patients (qPCR 1e+05, 1e+06). From the same model we estimated the odds ratios (OR) with 95% confidence intervals using the group-1 as the reference group. Flies fed on group-2 or 3 patients had a 3-5-fold greater chance of becoming infected. These findings confirm that VL-HIV patients have high parasitic burden and transmit infection to the vector.

Keywords XENODIAGNOSIS; VISCERAL LEISHMANIASIS; HIV; TRANSMISSION

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O24-04: PAEDIATRIC CUTANEOUS LEISHMANIASIS IN FRENCH GUIANA: EPIDEMIOLOGICAL VARIATIONS AND PENTAMIDINE EFFICIENCY

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American tegumentary leishmaniasis (ATL) is endemic in French Guiana. *Leishmania guyanensis* represents 80% of cases, which makes systemic pentamidine its first-line treatment. However, the management of ATL is mainly based on studies conducted in adults and limited data are available on the use of pentamidine in children. Our objectives were to describe the clinical and epidemiological characteristics of ATL in children in French Guiana and to evaluate the efficiency and safety of its treatment. A retrospective study was conducted, reviewing the records of all patients < 18 year-old with compatible lesions and at least one positive parasitological test between 2010 and 2020. In total, 105 children were included. Amerindians, Brazilians, Maroon, Creole and French metropolitans were equally represented (about 20% each). Median age was 11.5 (7-16). Boys were more numerous over the age of 15 years (sex ratio 4:1) but not under 15 years (1:1). The number of monthly cases showed a seasonal pattern with a peak in February and a nadir in September. The occurrence of familial cases was reported in 48.6% of children. *L. guyanensis* was the most frequent species (89/95 cases, 93,7%), followed by *L. braziliensis* (three cases) and *L. lainsoni* (two cases). The mean number of lesions was 2.3. The most frequent presentation was ulcerative (86,7%) with involvement of the lower limbs (58,1%). After first-line therapy (pentamidine in 74/75 cases),

38 children (52%) presented a complete response, 18 (24%) were lost to follow-up and 18 (24%) needed a new course due to stable or worsening lesions. Among them, two were switched to amphotericin B (pentamidine-induced hepatitis) or meglumine antimoniate (late isolation *of L. braziliensis*), while 16 received a new pentamidine course. Fourteen then presented a complete response while clinical failure was observed in two cases. Mild side effects were reported in 19 patients (26%), including headache, dizziness, nausea/vomiting and elevated CPK level. Most children received a single-shot IM injection (7mg/kg) while 10 were treated with the intravenous way (4mg/kd/d every two days, three injections). The incidence of ATL in children in French Guiana (about 10/year) is much lower than in adults (200/year) but remains noteworthy. Contaminations probably occur in different ways in teenagers and young children, as shown by the sharp difference in sex ratio. The masculine predominance over the age of 15 is probably explained by more outdoor exposures, while young boys and girls are similarly affected by indoor contaminations. The importance of indoor exposure is best highlighted by the frequency of familial cases. The predominance of Brazilian patients reported in adults was not observed in children. *L. lainsoni* was almost as frequent as *L. braziliensis*, suggesting the reservoir of this species might be found closed to human settlements. Concerning treatment, pentamidine was shown as a very efficient and safe therapeutic option. Intravenous injections are more comfortable in young children but a single IM injection can be useful in isolated. This work highlights different exposures to ATL in adults and children. Pentamidine should be advised as first-line therapy in settings where *L. guyanensis* is predominant.

Keywords CUTANEOUS LEISHMANIASIS; CHILDREN; PENTAMIDINE; FRENCH GUIANA

O24-06: "CHEAPER AND BETTER": AN ECONOMIC ANALYSIS OF CHANGING FIRST LINE TREATMENT FOR CUTANEOUS LEISHMANIASIS IN BOLIVIA

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Cutaneous leishmaniasis (CL) is endemic in Bolivia, mostly affecting poor people in rainforest areas. The current first-line treatment consists of systemic pentavalent antimonials (SPA) for 20 days and is paid for by the Ministry of Health (MoH). Long periods of drug shortages, a lack of conditions to deliver treatment safely, treatment interruption are challenges to implementation. Intralesional pentavalent antimonials (ILPA) are an alternative to SPA. This study aims to compare the cost of ILPA and SPA, and to estimate the health and economic impacts of changing the first-line treatment for CL in an endemic area of Bolivia. The cost per patient treated was estimated for SPA and ILPA from the perspectives of the MoH and society. The quantity and unit costs of medications, staff time, transportation and loss of production were obtained through a health facility survey (N=12), official documents and key informants. A one-way sensitivity analysis was conducted on key parameters to evaluate the robustness of the results. The annual number of patients treated and the budget impact of switching to ILPA as the first-line treatment were estimated under different scenarios of increasing treatment utilization using previous estimates of the extent of underreporting. Costs were reported in 2016 international dollars (1 INT$ = 3.10 BOB). Treating CL using ILPA was associated with a cost saving of $248 per patient treated from the MoH perspective, and $688 per patient treated from the societal perspective. ILPA was cost-saving even under a hypothetical increase of 80% in the number of cases treated. Switching first-line treatment would allow two-and-a-half times the current number of patients to be treated,

while maintaining the current budget. The results of this study support a shift to ILPA as the first-line treatment for CL in Bolivia and possibly in other South American countries

Keywords CUTANEOUS LEISHMANIASIS; ECONOMIC ANALYSIS; INTRALESIONAL PENTAVALENT ANTIMONIALS; BOLIVIA; COST ANALYSIS

O32-03: IS IMPACT INCREASES WHEN MORE THAN ONE INTERVENTION CLUBBED FOR *Phlebotomus argentipes* (DIPTERA: PSYCHODIDAE) SAND FLY CONTROL IN BANGLADESH: A CLUSTER-RANDOMIZED CONTROL TRIAL

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Visceral leishmaniasis (VL) known as kala-azar in the Indian sub-continent is a parasitic disease that affects rural poor communities and is one of the neglected tropical diseases. The female sand fly, *Phlebotomus argentipes* transmits the parasite, *Leishmania donovani* into the human body. Several studies have been conducted to identify various effective VL-vector control tools during the past decade. However, understanding of the additional benefit of the double intervention tools compared to the single intervention tool was unclear. Therefore, we aimed this study to explore the efficacy of double intervention tools compared to single intervention tools against sandfly reduction in Bangladesh. A cluster randomized controlled trial was conducted where 3079 houses from 11 villages were divided into 10 sections, each section with 6 clusters and each cluster having approximately 50 houses. There were nine intervention arms: (1) indoor residual spraying (IRS) with alpha-cypermethrin; (2) long-lasting insecticide-impregnated bed-net (LLIN); (3) impregnation of local bed-nets with slow-release insecticide KOTAB 1-2-3 (KOTAB); (4) insecticide spraying in the possible breeding places using chlorpyrifos (OUT) and their combinations, (5) IRS + LLIN; (6) IRS + KOTAB; (7) IRS + OUT; (8) LLIN + OUT; and (9) KOTAB + OUT

and a control arm. Vector density was the key outcome variable which was measured before and 2, 4, 5, 7, 11, 14, 15, 18, and 22 months after intervention using CDC light traps. At each follow-up point, the percent reduction of female *P. argentepis* sand flies in both single and combined intervention arms was calculated against the control arm and compared to understand the difference in the efficacy between single and double intervention. At baseline, the average female *P. argentepis* per household was 5.7 (SD=6.8) in the control arm, whereas it was 3.7 (SD=3.0) to 6.2 (SD=10.7) in the single intervention arms and 3.8 (SD=3.9) to 6.1 (SD=8.7) in the double intervention areas. There was no significant difference between control and intervention (single or double) arms. The mean reduction of female *P. argentepis* sand flies compared to the control arm was 70.2%; 59.6%; 56.5%; and 55.0% in the IRS, LLIN, KOTAB, and OUT arms, respectively. In contrast, it was 79.8%; 73.3%; 71.3%; 67.8%; and 39.1% in the double intervention arms IRS+LLIN, IRS+OUT, LLIN+OUT, IRS+KOTAB, and KOTAB+OUT respectively. The density of female *P. argentepis* sand flies was significantly reduced in the intervention arms compared to the control arm. Moreover, double interventions showed more female *P. argentepis* sand fly reduction than single interventions in most of the follow-up measurements. National programmes can get the additional benefit of vector reduction by implementing one of the combined intervention tools for controlling VL vectors.

Keywords VISCERAL LEISHMANIASIS; COMBINED VECTOR CONTROL TOOL; BANGLADESH; *Phlebotomus argentipes*

O32-04: NEW SCENARIOS OF CHAGAS DISEASE IN AREAS CERTIFIED WITHOUT *Trypanosoma cruzi* TRANSMISSION BY *Rhodnius prolixus* IN BOYACÁ, COLOMBIA

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Updating the distribution, natural infection status and potential risk of *Trypanosoma cruzi* transmission is critical for planning, prioritizing, and implementing strategies to control Chagas disease (CD), especially after *Rhodnius prolixus* control programs. Next implementation of control program, the Department of Boyaca contains the highest number of municipalities certified by PAHO to be free of intra-domestic *T. cruzi* transmission by *R. prolixus.* The present work describes the spatial distribution, natural infection (NI), blood meal determination and *T. cruzi* molecular characterization in synanthropic triatomines and domestic mammals from this Colombian region between 2017 to 2021. An entomological survey was conducted in 52 municipalities from Boyaca known to have had previous infestations of triatomine bugs. Insects were collected through active searches carried out by technical personnel from the Secretary of Health. The distribution of the collected triatomines was analyzed to identify any vector hotspots using spatial recreation. In addition, we designed a comprehensive, multi-faceted molecular study including: (*i*) blood meal source determination, (*ii*) *T. cruzi* infection rate in collected triatomines, (*iii*) identification of circulating *T. cruzi* genotypes and (iv) *T. cruzi* molecular diagnosis in domestic dogs. We identified the two infected vector species: (i) *Triatoma dimidiata* in the northeast and (ii) *Triatoma venosa* in the southwest region. A total of 90 *T. dimidiata* were collected, which *T. cruzi* infection was evidenced in 40% (36/90) of these triatomine bugs. Only DTU I was found, and TcI $_{Dom}$ was the most distributed. Blood-

meal analysis showed that *T. dimidiata* only fed of human blood. Seroprevalence in domestic dogs was 4.6% (3/66) and not *T. cruzi* DNA was found in these dogs. On the other hand, in southwest, 101 *Triatoma venosa* were collected in domestic and peridomestic habitats. A natural infection of 13.9% (14/101) was noted. Interestingly, four feeding sources were identified: humans, domestics dogs, *Rattus ratus,* and *Gallus gallus*. A high seroprevalence of 46.5% (40/86) was observed in dogs, and *T. cruzi* DNA was detected by PCR in 14 of them (16.4%). Only $TcI_{sylvatic}$ DTU was identified in infected dogs. **Conclusions:** After some municipalities were certified to be *R. prolixus* free, *T. dimidiata* and *T. venosa* have become the most significant insect vectors in the *T. cruzi* transmission. The molecular and spatial analysis used here allows us to identify areas with an ongoing threat of parasite transmission. *T. dimidiata,* is associated to domestic transmission but with the ability to connect both transmission cycles. In this scenery, domestics dogs have an insignificant role in local ecoepidemiology. Finally, *T. venosa* is associated with enzootic transmission and domestics dogs have an active role of reservoir in the local epidemiology.

Keywords EPIDEMIOLOGY; COLOMBIA; CHAGAS DISEASE; DOMESTIC DOGS; *T. dimidiata*; *T. venosa; R. Prolixus*

O32-05: CHAGAS DISEASE, CLINICAL AND MOLECULAR SURVEILLANCE IN RURAL AREAS EXPOSED TO TRIATOMINAE SPECIES AT COLOMBIAN CARIBBEAN, CHAGCOV PROJECT

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American Trypanosomiasis, Chagas disease (CD) caused by the protozoan parasite *Trypanosoma cruzi* represents a public health problem because it is part of the group of Neglected Tropical Diseases (NTD). Chagas disease affects annually approximately 6 to 8 million people worldwide, with an estimate 50.000 deaths; likewise, between 60 to 100 million people live in risk areas around the world including Colombian Caribbean region. Part of the response for the control of CD is made by research groups in Tropical Medicine of the Colombian Caribbean. This project proposed to perform a surveillance in molecular and clinical identification of CD at the human settlements exposed to Triatominae species, and Chagas disease in rural municipalities of the Atlantic and Bolívar, Colombia. This was a descriptive epidemiological study of prevalence with prospective data collection. Patients with suspected diagnosis or risk factor of Chagas disease were included due their environmental risk of exposure to Triatominae located in the department of Bolívar and Atlántico such as the municipalities of the study. A serum sample was taken and / or whole blood for the application of serological and molecular tests aimed to characterize patients as carriers or not of IgG antibodies against *T. cruzi*. The results showed the serological profile of the recruited patients in relation to Chagas disease for the first

time for these rural municipalities in Colombian Caribbean where the parasite and the transmitting vector of the disease has been described in the past. This information is important as a public health surveillance measure since, up to the date of this work, no cases of Chagas disease have been notified in government reports in the region of study, but there have been positive cases in neighboring municipalities therefore this work and its findings are a response to a population with many risk factors for the presence of this vector borne and neglected tropical disease in Colombia.

Keywords CHAGAS DISEASE; *Trypanosoma cruzi;* TRIATOMINAE SPECIES; COLOMBIA; NEGLECTED TROPICAL DISEASE

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O32-06: PROGRAMME-SCALE MOLECULAR SURVEILLANCE OF INSECTICIDE RESISTANCE IN *Phlebotomus argentipes* REVEALS SPATIAL-TEMPORAL TRENDS

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Validated diagnostic doses for susceptibility to insecticides in the primary visceral leishmaniasis vector *Phlebotomus argentipes* are currently undefined, which limits the effectiveness of using bioassays to monitor resistance, coupled with logistical challenges of phenotypic monitoring at a large scale. Molecular resistance diagnostics can be used to monitor phenotypic resistance at a wide scale, when relevant and informative markers are available. Previous indoor residual spraying campaigns in Bihar used DDT, which has now been superseded by a synthetic pyrethroid, both of which target the voltage gated sodium channel (Vgsc). Two knockdown resistance (kdr) mutations (L1014F and S) are found in the Vgsc of *P. argentipes* in India, which confer resistance to DDT and pyrethroids. This study aimed to monitor spatial variation and temporal changes in kdr in Bihar and also to investigate possible links between changes in resistance sand fly populations, with the frequency of IRS spraying. Sand flies were collected monthly across 8 Bihar districts were analysed for kdr mutations using two qPCR assays to estimate levels of resistance in each region. The resistance was then compared to IRS spray data for surrounding villages. Spatial variation in kdr was pronounced among districts, but most showed a consistent increase in resistant alleles from 2017 to 2018, especially in the more strongly-resistance linked phenylalanine (1014F) kdr allele. However, from 2018-2021 resistance frequencies stabilised. There was no simple relationship evident between the recent or past history of spraying and kdr frequencies, and further investigation of the driver of the increase is required. Whilst the increase in kdr frequency is concerning, the

stabilization coupled with the absence of known operational level resistance to full dose pyrethroids to date is encouraging. Nevertheless, careful monitoring of the quality of IRS spray is crucial, and consideration of alternative insecticides with a different target site is important for sustainability.

Keywords KDR; INDIA; RESISTANCE; IRS; ALPHA-CYPERMETHRIN

O39-01: MODELLING SPATIOTEMPORAL PATTERNS OF VISCERAL LEISHMANIASIS INCIDENCE IN INDIA USING ENVIRONMENT, BIOCLIMATIC AND DEMOGRAPHIC DATA, 2013-2021

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Visceral leishmaniasis (VL) is a vector-borne disease caused by *Leishmania donovani* and transmitted through infected female *Phlebotomus argentipes* sandflies. In India, VL has been endemic in 633 subdistricts (blocks) spread over 54 districts in four states (Bihar, Jharkhand, Uttar Pradesh and West Bengal) affecting nearly 150 million people. As of 2020, the National Kala-azar Elimination Programme has achieved VL elimination (<1 case / 10,000 population/year in each 'block') in 596 blocks. There is a need for sustaining the elimination level in these blocks, while a more targeted approach is necessary to achieve the WHO 2030 target in those blocks where elimination has not been reached. In an earlier publication, applying a statistical model on surveillance data collected from the states of Bihar and Jharkhand in India, we forecasted monthly VL incidence at the block level. The model predictions may be used to help the programme for logistics management in advance. In this study, we have improved the predictive power of the model incorporating (i) environmental, bioclimatic and

demographic factors that influence VL transmission dynamics, and (ii) spatial, temporal and spatiotemporal random effects to minimize the variability unexplained by the above factors. We modelled the spatiotemporal distribution of reported VL cases for a 9-year period (2013-2021) in the states of Bihar and Jharkhand and its association with environmental, bioclimatic and demographic factors using non-parametric models with space-time interactions. A negative binomial distribution was assumed to describe the block level monthly VL cases. Initially, we fitted 46 models to a training data set (2013-2018) using the Bayesian inference via Integrated Nested Laplace Approximation (INLA) approach. The best fitting model was selected based on deviance information criterion (DIC) and was validated with a test data set (2019-2020). The model was further used to forecast VL incidence beyond the period of observations (2021-2022). We found that minimum temperature, enhanced vegetation index, population density and, isothermality played a positive role in VL occurrence. Conversely, precipitation, maximum temperature and soil moisture were negatively associated. During both training and testing periods, model predictions agree with the observed declining trends in many blocks both above and below the elimination threshold. Predictions beyond the period of observations (2021-2022) showed that the annual incidence is more likely to exceed the elimination threshold in the blocks where the reported VL incidence was > 6 per 10,000 population in 2013. Our spatiotemporal modelling framework with environmental, bioclimatic and demographic factors could better explain spatiotemporal patterns in VL incidence at block level and therefore may be used to forecast trends in incidence during post-elimination. Model predictions for 2022 highlighted the need for targeted control measures in blocks where the annual incidence was > 6 per 10,000 population in 2013 to achieve elimination

Keywords KALA-AZAR; LEISHMANIASIS; SPATIOTEMPORAL TRANSMISSION; FORECASTING; INDIA

Financing Bill and Melinda Gates Foundation supported the study via SPEAK India consortium (OPP1183986)

O39-02: VISCERAL LEISHMANIASIS: TRANSMISSION ASSESSMENT IN NON-ENDEMIC DISTRICTS IN WESTERN NEPAL

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Visceral leishmaniasis is on the verge of elimination as a public health problem (target annual incidence of <1/10,000 population) in the Indian subcontinent. Since the start of the elimination initiative in 2005, there has been a strong reduction in reported VL cases, and Nepal was the first country in the region to reach the elimination target in 2013. However, increasing numbers of VL cases are being reported from districts that are not known to be endemic. In 2019, an independent assessment of the VL elimination progress made the recommendation to assess the true extent of endemicity in Nepal. A multidisciplinary survey was conducted during 2018-2019 to collect epidemiological, entomological and microbiological data on *Leishmania donovani* infection in seven officially non-endemic districts to assess the possibility of local transmission and guide policy in Nepal. An exhaustive house-to-house survey was conducted in 19 villages to collect information on demographic characteristics, VL-related events, and travel exposure to VL endemic areas in the past two years. All individuals aged ≥ 2 years who lived permanently in the villages were invited to provide a 2ml venous blood sample which was used for rK39 RDT testing on the spot and distributed in an EDTA tube, a serum tube and onto a pre-printed

Whatman # 3 filter paper to assess the sero-prevalence of *L. donovani* infection through serological testing (Direct Agglutination Test) and kDNA PCR. In parallel, sand flies were captured indoors in 12 households in each study village for two consecutive nights, using CDC light traps in combination with mouth aspiration. Sandflies were morphologically identified and molecular analysis was done to assess *leishmania* infection and confirm the sand fly species. Our survey retrospectively documented 28 cases of past (treated) VL cases, of whom only six reported a travel history outside of the district in the two years prior to the diagnosis. The median age at time of diagnosis was 27.5 years (Q1=16.5 years, Q3=42.2 years). In addition, six new active VL cases were identified. Overall, 2.0% of the study population (23/1355) was positive for *Leishmania* infection with rK39 RDT after exclusion past and current VL cases, varying from 1% and 3% in the different survey villages. Median age of RDT positive individuals was 15 years (Q1=12.5 years, Q3=23.5 years), 60.9% were male. DAT was positive in 47/1354 (3.0%) persons without past or current VL; varying between 2% and 4% in villages. Median age of DAT positive individuals was 16 years (Q1=11.5 years, Q3=23.5 years), 59.6% were male. Leishmania species was detected in 7/996 (1%) samples, ranging between 0% and 3% for villages. The sand fly vector *Phlebotomus argentipe*s was captured in all except one survey districts. Molecular analysis to identify *leishmania* infection in the sandflies is in progress. Our findings show evidence for low grade local transmission in VL in the survey villages. The VL elimination initiative in Nepal should consider extending its surveillance and control activities, and redesigning the risk map for VL in Nepal.

Keywords *Leishmania donovani*; rK39; DAT; *Phlebotomus argentipes*

O39-03: ACHIEVING THE ZERO TRANSMISSION OF KALA-AZAR IN BANGLADESH BY 2030: AN EPIDEMIOLOGICAL ASSESSMENT

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Bangladesh, India, and Nepal were jointly signed a memorandum of understanding (MoU) in 2005 to eliminate the kala-azar from their respective countries, which was later extended to 2017. The elimination target was set to reduce cases to less than one per 10,000 population at Upazila (sub-district) level. The country reached its target in 2016. The present objectives of the country are to obtain the elimination certificate from World Health Organization by 2022 and achieve zero transmission and kala-azar free Bangladesh by 2030. Therefore, we aimed to assess the epidemiological data to determine whether it is feasible or not with the current trend of reduction of cases. We have collected the web-based real-time disease surveillance (DHIS2) data from 2015 to 2021, and Upazila wise aggregated data for 2008 and 2014 from the National Kala-azar Elimination Program (NKEP), Communicable Disease Control (CDC) unit of the Directorate General of Health Services (DGHS). A total of 22,652 cases, including 68 deaths, were reported from 177 Upazilas under 51 districts between 2008 and 2021. Officially, Bangladesh has 100 program Upazilas where all interventions were provided; however, cases from non-program

Upazilas have received diagnosis and treatment facilities free of cost. Mymensingh is one of the highest endemic districts and reported 64.54% (14,620) patients compared to the total caseload. Fulbaria and Trishal Upazilas were the most endemic Upazilas and reported 5260 and 5144 cases. The highest incidence rate was 7.25/10,000 population in 2014, which came down to 1.57 in 2015 and above the elimination target in 14 and four Upazilas, respectively. Finally, the country reached the elimination target in 2016 in all Upazilas of below one case per 10,000 population and the total reported cases was 454. Bangladesh reported 380, 290, 224, 132, and 99 cases between 2017 and 2021. The number of cases will be about 'zero' in 2030 if we consider the last five year's (2017-2021) average reduction of 36.6%. Several interventions include sensitization of communities for seeking health care and vector control, training of health care professionals, early diagnosis and effective treatment including 12 months post-treatment follow-up, integrated vector management focusing on indoor residual spraying, and effective disease surveillance using DHIS2. Bangladesh will be a kala-azar free country by 2030 when all interventions are continued.

Keywords ZERO TRANSMISSION; ELIMINATION CERTIFICATE; INTERVENTIONS; POST-TREATMENT FOLLOW-UP; VECTOR CONTROL

O39-04: COMBINING HUMAN SEROSURVEILLANCE WITH MOLECULAR XENOMONITORING IN TWO ENDEMIC VILLAGES FOR VISCERAL LEISHMANIASIS SURVEILLANCE IN SARAN, BIHAR, INDIA

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As India prepares for post-elimination of visceral leishmaniasis (VL), it will become much harder to detect human cases, especially if case numbers decrease further or occur in new areas with limited or no surveillance activity. New strategies for surveillance are required, monitoring transmission rather than disease, and targeting both humans and the sand fly vector, *Phlebotomus argentipes*. In 2019, researchers in the SPEAK India molecular xenomonitoring (MX) and surveillance workpackages focused VL detection efforts in endemic villages of Bishambharpur and Rampur Jagdish (having reported VL cases in each of the 3 years prior to the study, i.e. 2016-2018) in the Saran district of Bihar, India. The goal of this work was to assess whether a combined human serological and MX approach allows for detection of ongoing VL transmission. For MX, field teams set 10 CDC light traps in each village every fortnight in households that consented to participate in the study from June to September 2019. Sand flies were first sorted from other vectors (i.e. mosquitoes) then by sex and genus and stored at -20 C. They were later dissected and stored as individual heads and pooled thorax-abdomen sections grouped by date and village of collection. Upon DNA extraction, a quantitative polymerase chain reaction assay targeting the conserved REPL repeat region repeats of *Leishmania donovani* was used to detect infection if Ct values were ≤ 30. For serosurveillance, all

inhabitants of the two villages ≥2 years old were requested to provide a capillary blood sample for testing with the rK39 Rapid Diagnostic Test (RDT) on the spot, and collection on a filter paper for testing with Direct Agglutination Test (DAT) and rK39 ELISA. Demographic and VL-related events were collected for each inhabitant. Overall and age group specific seroprevalences were calculated per test. A total of 727 females *P. argentipes* in 79 pools were analyzed. No pools were positive for *L. donovani*. Blood samples were provided by 2756 (76%) inhabitants of Bishambharpur and 2773 (74%) of Rampur Jagdish. Both villages had 35 participants reporting a VL history. RDT and DAT seroprevalence was relatively low in all age groups in both villages (<2%). ELISA seroprevalence on the other hand was high (>8%) in all age groups in Bishambharpur, which had continuous cases during and after the study period. This in contrast to Rampur Jagdish which showed ELISA seroprevalence <2.5% in all age groups, but did not have any new VL cases reported since the start of the study. Though no positive sandfly pools were detected, serosurveillance was able to detect current infections in the two villages and seems the more sensitive approach in detecting VL transmission. The rK39 ELISA seemed to reflect current transmission well, although this finding is based on high ELISA seroprevalence in one village only. More frequent and targeted sand fly collection events may have resulted in increased infection detection, specifically if traps were set in households of infected inhabitants. This combined approach may be useful in confirming focal transmission sites during the elimination phase of VL.

Keywords MOLECULAR XENOMONITORING; VL DETECTION; SEROSURVEILLANCE; VISCERAL LEISHMANIASIS; VECTOR SURVEILLANCE

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O39-05: CONTRIBUTIONS OF THE SYNDEMIC THEORY TO THE APPROACH TO CUTANEOUS LEISHMANIASIS IN HIPERENDEMIC AREAS IN BAHIA, BRAZIL

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This study analyses the contributions of the syndemic approach to orient coordinated interventions against cutaneous leishmaniasis (CL) in endemic areas. In the scientific literature there is a huge production on the pathophysiological, epidemiological, clinical aspects, as well as the treatment, the control and prevention strategies measures, which consolidates a robust understanding of the biomedical phenomena of LC. Only a small number of studies addressed the relationships of LC with social determinations and other diseases which permit us to consider the holistic conceptualization of health, the social character of the health-disease and care processes, and the construction of public policies to ensure the right to health. To implement the knowledge about these interactions is essential to overcome the centrality of the biomedical care model as well as to guide the health services to structuring the care for people with LC. The syndemic approach enables us to increase the understanding of these interactions and to strengthen the link between the biomedical perspective of disease development and the wide-ranging social forces that influence the occurrence and aggravate LC. Syndemia is understood as a process of synergistic interaction between one or more diseases, in which their effects are mutually potentiated. The adoption of this concept emerges from the observation and immersion in loco in an endemic area in Brazil, in the municipality of Valença, in Bahia. This study is part of the project "ECLIPSE-

Empowering people with Cutaneous Leishmaniasis". An international research and intervention programme developed in three countries impacted by LC: Sri Lanka, Ethiopia and Brazil, coordinated by academic research institutions from the respective countries and the UK. The project assumes cartography as a method to approach the territory by draw lines, mapping, following the movements, connections and flows of the LC care networks. Taking vulnerabilities and care itineraries in rural communities as the central axis, it is noticeable that the confrontation of LC cannot be reduced to a focus on the disease itself, shaped by a biomedical perspective. LC affects mostly black people, with low income and education, impacted by social exclusion and the absence of public policies in the communities. The social, economic and environmental context potentiate the interaction between LC and coexisting diseases, such as diabetes, hypertension, AIDS/HIV, obesity, cancer, alcoholism and heart disease, and amplify their effects. Even if these interactions do not occur at the biological level, the convergence of diseases and the interconnection with the processes of vulnerability, racism and stigma, result in the potentiation of psychic suffering and compromise the general health status of the person with LC. A syndemic approach is useful for the analysis of the synergistic interactions between diseases and the contexts that exacerbate them. It permits consequently to improve the implementation of comprehensive, integrated and intersectoral public policies that can are more appropriate for the local reality considered in its complexity. Considering this scenario, we recommended reorienting the strategies against LC for a comprehensive care by structuring care networks that permits to consider the collective and individual demands of the rural communities.

Keywords SYNDEMIA; CUTANEOUS LEISHMANIASIS; SOCIAL DETERMINATION OF HEALTH; HEALTH POLICY

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O39-06: USE OF SOIL MOISTURE ACTIVE PASSIVE SATELLITE DATA (SMAP) AND WORLDCLIM 2.0 DATA TO PREDICT THE POTENTIAL DISTRIBUTION OF VISCERAL LESIHMANIASIS AND ITS VECTOR LUTZOMYIA LONGIPALPIS IN SÃO PAULO AND BAHIA STATES, BRAZIL

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Visceral leishmaniasis (VL) is a neglected tropical disease transmitted by Lutzomyia longipalpis, a sand fly species widely distributed in Brazil. Despite efforts to strengthen national control programs for VL, reduction in its incidence and geographical distribution in Brazil is still a challenge. VL is re-emerging and expanding its range to urbanized areas. Ecological niche models (ENM) for use in surveillance and response systems may enable more effective operational VL control by mapping risk areas and elucidating eco-epidemiologic risk factors. ENMs for VL and Lu. longipalpis were generated using monthly WorldClim 2.0 data and monthly SMAP L4 soil moisture data. SMAP L4 images from day 1 and day 15 for each month were selected. ENM were developed using MaxEnt software to generate risk maps based on an algorithm for maximum entropy. The jackknife procedure was used to identify contribution of each variable to model performance. The three most meaningful components were used to generate ENM distribution maps in ArcGIS 10.6. Similar patterns of VL and vector distribution were observed using SMAP as compared to WorldClim 2.0 models based on temperature and precipitation data or water budget. Cases of VL and known locations of Lu. longipalpis presented similar ENM. A unique match of the VL niche and the surface soil moisture measured by SMAP was observed and defined seasonality regarding soil moisture and precipitation data. Results indicate that direct earth observing satellite measurement of soil moisture

by SMAP can be used in lieu of models calculated from classical thermal and precipitation climate station data to assess VL risk.

Keywords LEISHMANIASIS; Lutzomyia longipalpis; SMAP; ECOLOGICAL NICHE MODEL; WorldClim 2.0

Financing NASA, USA

4.5 IMMUNOLOGY - CELL BIOLOGY – PATHOGENESIS - VACCINES

O3-02: PROGRESS TOWARDS DEVELOPING A SAFE AND EFFICACIOUS PAN *Leishmania* VACCINE

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Leishmaniasis is a neglected tropical disease with significant morbidity and mortality. Currently, there is a lack of an effective strategy to control this disease and achieve elimination by 2030, the target set forth by WHO. Vaccination can be an effective measure to control this disease and has the potential to achieve its elimination. Previously, efforts focused on the development of vaccines from killed parasites with or without adjuvants, subunit vaccines, and DNA/RNA based vaccines that were immunogenic and efficacious in animal models against virulent infection through needle

injection. However, when some of them were tested against natural sand fly infection or in clinical settings, they were determined not to be efficacious. People who have recovered from leishmaniasis are protected for life against future infections, suggesting that an effective vaccine is possible against this disease. Exposure to, or deliberate infection with wild type *Leishmania major* (leishmanization), was shown to be effective against both visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL). However, the practice of deliberate infection with the wild type parasites raises safety concerns and has not been pursued. Therefore, a well characterized genetically modified parasite that is safe, but equally efficacious as leishmanization, could be an alternative. The presence of both CL and VL in many of the endemic countries makes it desirable to have a vaccine that can protect against both forms of the disease. We have developed a vaccine candidate that has been genetically modified by deleting centrin gene, an essential gene for cell division for amastigotes, using CRISPR-Cas technology in a dermotropic *Leishmania major* parasite strain (*LmCen-/-*). We have shown that immunization with *LmCen-/-* parasites is safe and efficacious in appropriate animal models for both CL and VL against both needle and sand fly vector mediated virulent infection. Additionally, in animal models of CL, we have demonstrated that immunization with *LmCen-/-* induces effector and memory T cell responses comparable to leishmanization and equivalent protection against virulent infection. Further, the *LmCen-/* parasite vaccine manufactured in a bioreactor under Good Laboratory Practice, is safe, immunogenic, and provides long-term protection against both CL and VL in sand fly vector mediated challenge in preclinical studies. We have also demonstrated that GLP grade *LmCen*$^{-/-}$ parasites can elicit pro-inflammatory immune responses in peripheral blood mononuclear cells isolated from healthy individuals from nonendemic as well as from healthy, asymptomatic and VL healed individuals from endemic region suggesting that *LmCen-/-* parasite vaccine has the potential to induce a protective immune response in humans. In addition, we are developing biomarker of correlates of protection for *Lmcen-/-* parasites using *Leishmania donovani* total antigens under cGMP for *Leishmania* Skin Test that could be used in clinical trials. Currently, the *LmCen-/-* parasite vaccine is being manufactured under cGMP conditions for future clinical trials. Taken together, these results suggest

that a genetically modified parasite could be a viable vaccine candidate for evaluation in clinical trials.

Keywords PAN LEISHMANIA LIVE ATTENUATED VACCINE; SAFE AND EFFICACIOUS; SANDFLY CHALLENGE; CGMP MANUFACTURING

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Communication Disclaimer: My contributions are informal communication and represents my own best judgement. These comments do not bind or obligate FDA

O3-06: *Leishmania major CEN-/-* AS A EUKARYOTIC ANTIGEN DELIVERY SYSTEM: A POTENTIAL VACCINATION AGAINST SARS-CoV-2

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Leishmania major Friedlin *centrin*-knockout (*Lmcen-/-*) has been previously proved to be a safe, strongly immunogenic, and efficient live-attenuated vaccine against multiple strains of *Leishmania* and is soon to enter to the clinical trials phase. The avirulent *Lmcen-/-* only undergoes limited replication in the infected host but still generates a protective immune response. Unlike commonly used expression systems such as bacteria, yeast, plant or insect cells to produce recombinant proteins, *Leishmania* parasites generate proteins with glycosylation patterns similar to mammalian cells. Proper glycosylation of recombinant vaccine antigens is crucial for the effective T-cell priming and activation. Therefore, we genetically modified *Lmcen-/-* parasites to express viral antigens, for the purpose of delivering those antigens to the host to generate a dual immune response against both *Leishmania* and the viral antigens. *Lmcen-/-* was transfected with the commercially available plasmid pLEXSY containing genomic sequences that code for viral proteins. As a proof of concept, we expressed the S1 subunit and the Receptor Binding Domain (RBD) of the Spike protein of SARS-CoV-2. Both coding sequences were optimized for *Leishmania* transcription and were cloned into the pLESXY to allow either cytosolic expression of the protein or its secretion. After antibiotic selection and characterization, four clones of *Lmcen-/-* mutants were obtained: S1 cytosolic (LmcS1cyt), S1 secretory (LmcS1sec), RBD cytosolic (LmcRBDcyt) and RBD secretory

(LmcRBDsec). Female BALB/c mice were immunized with the recombinant parasites and non-transfected *Lmcen-/-* as a control. While none of the mice developed any symptoms of *Leishmania* infection, such as a cutaneous lesion, all four clones generated a strong T-cell immune response. To characterize the specific T-cell response against human Spike protein, we cultured *ex-vivo* mouse splenocytes with Hexa-pro Spike protein produced in human cell lines. T-cells isolated from LmcRBDcyt and LmcRBDsec immunized mice showed proliferation of IFN-γ-producing CD8+ T-cells specific for Hexa-pro Spike protein and low numbers of IL-4+ CD4+ and in IL-10+ CD8+ T-cells. In the case of mice immunized with S1-transfected parasites, there was a strong IFN-γ production in CD8+ and CD4+ T-cells only in LmcS1cyt immunized group, but not on those injected with LmcS1sec. Interestingly, these T-cell responses are only observed in when S1 antigen is delivered to the mice using the transfected *Leishmania,* but not when the recombinant Spike antigen is injected directly. These results demonstrate how *Lmcen-/-* can be used as a heterologous antigen expression system, and that the antigens produced by the parasite are similar to human antigens to generate T-cell immunity. Such antigens can induce strong IFN-γ production by T-cells, which is essential for anti-viral immunity. Further experiments will determine the efficacy of this protective immunity against a viral challenge and the potential of *Lmcen-/-* as a vaccine for other diseases.

Keywords *L. major CEN-/-; LEISHMANIA* DELIVERY SYSTEM; SPIKE PROTEIN SARS-COV-2

O4-01: GLYCOCONJUGATES (LPG/GIPLS) FROM DERMOTROPIC AMAZONIAN LEISHMANIA SPECIES DISPLAYS INTERSPECIES VARIATIONS IN THEIR BIOCHEMICAL AND FUNCTIONAL PROPERTIES IN C57BL/6 MACROPHAGES

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Lipophosphoglycans (LPGs) and Glycoinositolphospholipids (GIPLs) are multivirulence Leishmania glycoconjugates involved in the host-parasite interaction not only in the vector, but also in the vertebrate host. Information on the glycobiology of dermotropic Amazonian Leishmania species is scarce. For this reason, the objectives of this project were to report the biochemical and functions properties of LPGs and GIPLs. The species included: Leishmania braziliensis, Leishmania guyanensis, Leishmania shawi, Leishmania lansoni, Leishmania lindembergui and Leishmania naiffi. After extraction and purification of LPGs and GIPLs, the preliminary biochemical structures were obtained using fluorophore-assisted carbohydrate electrophoresis. Interesting polymorphisms were observed in the LPGs and GIPLs of the six species. Most of the LPGs were devoid of sidechains (type I) showing the typical Gal-Man-PO_4 repeat units. Those included L. shawi, L. lainsoni, L. lindembergui and L. naiffi. LPGs of L. braziliensis and L. guyanensis showed galactose and glucose sidechains, respectively. GIPLs from dermotropic Viannia species also had variations in their monosaccharide content. GIPLs from L. guyanensis and L. naiffi possesses galactose and mannose (Type II/hybrid), whereas for the other species, galactose was the main sugar (Type I). Those glycoconjugates were assayed in mouse peritoneal macrophages and respective TLR2 -/- and TLR4 -/- knock-outs for NO and cytokine/chemokine production. Regardelless the species, NO and cytokine/chemochine production were primarily via TLR4/TLR2. A higher pro-inflammatory activity was detected in L. lainsoni (type I LPG). We did not establish any correlation between

biochemical structure and functional activity. In conclusion, there are several biochemical polymorphisms in the LPGs and GIPLs of Amazonian dermotropic Viannia species. Those glycoconjugates were able to trigger variable innate immune responses in macrophages and may contribute to the spectrum of clinical manifestations in dermotropic Viannia species.

Keywords LIPOPHOSPHOGLYCAN; GLYCOINOISTOLPHOSPHOLIPIDS; VIANNIA; INNATE IMMUNITY; HOST-PARASITE INTERACTION

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O4-03: INFLUENCE OF DERMAL IMMUNITY FOLLOWING EXPOSURE TO SAND FLY BITES ON *Leishmania* INFECTION

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Leishmaniasis is a significant public health problem in many regions of the world. During acquisition of a blood meal, sand fly introduce an array of pharmacologically active salivary proteins and microbiota into the skin that influence hemostasis and inflammation. While it is known that these responses can influence the outcome of *Leishmania* infection, the immunological mechanisms underpinning this phenomenon and the immune response elicited by blood feeding are poorly understood. In the present study we investigated the immune response elicited by exposure to the bites of *Lutzomyia longipalpis* sand flies. As previously shown, short-term exposure to insect blood feeding activated salivary antigen-specific interferon (IFN)-gamma producing dermal-derived $CD4^+$ Th1 cells. However, upon repeated exposure, the immune response underwent diversification at the population level to include multiple salivary antigen-specific $CD4^+$ subsets (Th1, Th2, Th17 and T_{REG}), at both the dermal site of exposure and systemically. Analysis of the development of delayed type hypersensitivity (DTH) at the bite site during ongoing chronic exposure to sand fly bites revealed four phases of bite-induced DTH, the last of which correlated with a high-degree of immunoregulation. Chronic exposure was associated with enhanced cellular recruitment to the skin, an alteration in the maturation of inflammatory monocytes towards an alternatively activated macrophage phenotype, and enhanced disease upon subsequent challenge with *Leishmania* plus salivary gland homogenates. These observations demonstrate how exposure to sand fly blood feeding can alter the dermal environment and how the 'host-vector-pathogen' relationship

may impact the success of prophylactic and therapeutic intervention strategies against leishmaniasis.

Keywords LEISHMANIASIS; SAND FLY; SALIVA; HOST IMMUNE RESPONSE; SKIN

O4-04: SKIN PARASITE BURDEN AND SYSTEMIC INFLAMMATORY IMMUNE RESPONSE MOST CORRELATED WITH INFECTIOUSNESS TO SAND FLIES

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Phlebotomine sand flies maintain zoonotic transmission of parasites between dogs and humans. What makes a particular host infectious to sand flies is poorly understood. We examined immune responses via flow cytometry and infectiousness of dogs infected with *Leishmania infantum* from multiple clinical states to *Lutzomyia longipalpis* using xenodiagnosis. We found that dogs were infectious to sand flies at differing rates based on stage of infection, which also paired with more inflammatory T cell based immunity. Dogs with mild to moderate disease were significantly more infectious to sand flies than dogs with severe disease, high organ burden and a more regulatory immune response. We documented a substantial parasite burden in the skin of vertically infected dogs by RT-qPCR. There was a highly significant correlation between skin parasite burden at the feeding site and sand fly parasite uptake. This suggests dogs with high skin parasite burden and an Th1-based inflammatory immune response contribute the most to the infected sand fly pool. Although skin parasite load and parasitemia correlated with one another, the average parasite number detected in skin was significantly higher compared to blood in matched subjects. Thus, dermal resident parasites were infectious to sand flies from dogs without detectable parasitemia. Together, our data implicate skin parasite burden and earlier clinical status and an inflammatory systemic immune response as stronger indicators of outward transmission potential.

Keywords *Leishmania infantum;* RT-qPCR; *Lutzomyia longipalpis*

O4-05: INVESTIGATING THE ROLES OF TRIM24 IN MACROPHAGE ACTIVATION DURING VISCERAL LEISHMANIASIS

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Macrophages play a key role in the killing of intracellular pathogens, but in many cases, they can also provide a niche where pathogens survive and even replicate. *Leishmania* parasites can both persist and replicate in their macrophage hosts. These parasites have evolved several strategies to counteract their host cell's antileishmanial response including modulation of the macrophage signalling pathways. The mechanisms by which these signalling pathways are regulated by the macrophage and potentially manipulated by the parasite remain poorly understood. Recently TRIM24, a member of the tripartite motif protein family, was predicted as an upstream negative regulator of host pro-inflammatory gene expression in a murine model of VL. In this study we utilize *Trim24*-deficient C57BL/6 mice to investigate the roles of TRIM24 in macrophage activation *in vitro* and *in vivo*. Comparing bone marrow-derived macrophages (BMMs) from B6.*Trim24*$^{-/-}$ and wild type B6 mice we found that *Trim24*-deficiency was associated with increased expression of iNOS and release of nitric oxide. Despite the important role of NO in host protection, we did not observe any difference in parasite loads *in vivo*, as determined by bioluminescence imaging of *L. donovani*-infected B6 and B6.*Trim24*$^{-/-}$ mice. To further understand why *Trim24*-deficiency fails to lead to improved parasite killing (through elevated NO) we examined release of IFN-β, implicated in the negative regulation of immunity to *L. donovani*. BMMs from B6.*Trim24*$^{-/-}$ mice produced more IFN-β in response to LPS compared to wild type BMMs. Collectively, our data provide a potential mechanism whereby the positive (NO) and negative (IFN-β) effects of *Trim24*-deficiency are counter-balanced in vivo.

Keywords MACROPHAGE; TRIM24; LEISHMANIA; VL

O4-06: ORAL AND INTRAGASTRIC: NEW ROUTES OF INFECTION BY *Leishmania infantum* AND *Leishmania braziliensis*

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Infection by *Leishmania* parasites may result in diseases with significant clinical and epidemiological diversity. Although transmission in nature is related as exclusively occurring through the bite of an infected sand-fly vector, other possible transmission routes are speculated to occur, such as oral route, which are the subject of this work. We evaluated the possibility of infection by this route in Golden hamsters (*Mesocricetus auratus*) using *Leishmania braziliensis* (Lb) and *Leishmania infantum* (Li) parasites. Hamsters were separated into groups and infected with 10^6-10^7 *L. braziliensis* and *L. infantum* parasites as described: Lb1/Li1 were the positive control groups infected intradermically or intraperitoneally, respectively; Lb2/Li2 were intragastrically infected with promastigote forms derived from axenic culture; Lb3/Li3 were orally infected with promastigote forms derived from the same axenic culture; Lb4/Li4 were orally infected with a suspension of cultivated macrophages infected by *L. braziliensis* or *L. infantum* amastigotes; Lb5 were orally infected with *Lutzomyia longipalpis* which had fed directly on skin lesions from animals of group Lb1; Lb6 were orally infected with a fragment of the dermal lesion from animals of group Lb1; and Lb7 were orally infected with fragment of

the spleen with macroscopic lesions from an animal of group Lb2. After 120 days, animals were euthanized for parasitological, serological, and molecular analysis. Besides positive PCR reactions (Multiplex PCR targeting the *Leishmania* sp. kDNA), isolation of parasites in culture medium confirmed the infection in skin, spleen, and liver fragments from animals belonging to groups Lb1, Li1, Lb2, and Li2. The results obtained with the Lb2/Li2 groups confirmed our hypothesis and hamsters became infected after intragastric inoculation of promastigote forms. This infection route may occur in nature due to the insectivorous habit of some mammals, which can end up ingesting infected sandflies. Group Lb4, which ingested culture of macrophages infected with *L. braziliensis* amastigotes, also presented positive results in Multiplex PCR for spleen, liver, and stomach, besides positive Immunofluorescence Antibody Test (IFAT). These results also confirmed our hypothesis that amastigote forms inside macrophages can infect a mammal host when orally ingested, reflecting what may happen in nature when carnivores prey infected small mammals. Negative results were observed in animals from groups Lb3, Li3, Li4, Lb5, Lb6, and Lb7. Many factors can be involved in the absence parasites' detection in these groups, such as peculiarities of the parasites' strains or individual features of the employed outbreed experimental model. Defining the viability of *Leishmania* sp. parasites to be orally transmitted is essential to understanding their nature transmission dynamics. To the best of our knowledge, this is the first time that oral and intragastric transmission of *Leishmania* parasites was experimentally demonstrated, constituting new routes of infection, at least for *Leishmania infantum* and *Leishmania braziliensis.*

Keywords INTRAGASTRIC/ORAL TRANSMISSION; HAMSTERS; *L.infantum; L. braziliensis*

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O10-01: EVALUATION OF INHIBITION OF ROCKII BY SELECTED miRNA IN *Leishmania major* INFECTED THP1 CELLS.

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Intracellular pathogens such as *Leishmania major* adopt various strategies to circumvent immune defenses and proliferate inside the cells they infect. *L.major* has a large effector proteins, which are used by the pathogen to manipulate host signaling pathways to the pathogens advantage. MicroRNAs (miRNAs), small non-coding RNAs with evolutionarily conserved sequences, are expressed in various tissues and cells that play key part in various physiological and pathologic processes. Increasing evidence implies roles for miRNAs in microbiol infectious diseases by modulating inflammatory responses, cell penetration, tissue remodeling, and innate and adaptive immunity. Harnessing of dys-regulated miRNAs in parasite infection may be an approach to improving the diagnosis, prevention and therapy of infectious diseases. Rho kinases (ROCKs) belong to the serine–threonine family, the inhibition of ROCKs would affect the function of many pathways. Thus , ROCK inhibitors have potential therapeutic effects in a wide variety of pathological conditions from asthma, to cancer. In macrophages, inhibition of ROCK2 by selected miRNA would induce alterations in the mRNA, miRNAs and enzymatic profiles that lead to the control of infection. In this study , we experiment the first step of *Leishmania*-host (macrophage) interaction, focusing on the IL-10 expression and arginine and iNOs measurments pointing to possible targets to be used for the control of the infection. The arginase and nitric oxide synthase-like, have essential roles in the parasite survival and in the maintenance of infection. Hence, this study focus on the ROCK2 inhibition by selected anti-ROCK2 miRNA in macrophages which are the first defense cells in *Leishmania* pathogenesis. The objective of this study was to evaluate the ROCK II inhibitor activity by selected miRNA , which has been choosed

by bioinformatic analysis and the miRNA has been documented as ROCK II inhibitor. The differential expression of selected miRNA in macrophages was compared under two conditions: control (MQ + Parasite) and (MQ transfected with synthetic miRNA +parasite) for 5 hr and 24hr after Leishmania infection. The result shows inhibition of ROCK II kinase could modulate the proliferation and survival of parasites .

Keywords ROCKII; *Leishmania*; MIRNA

O10-03: EXTRACELLULAR VESICLES RELEASED FROM *Leishmania* INFECTED CELLS (LIEVS) INDUCE MACROPHAGE POLARIZATION TO THE M2 TYPE IN INFECTED TISSUES

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Visceral leishmaniasis is a neglected tropical disease that causes significant morbidity and mortality. Iconic images of the distended abdomen of infected individuals due to an enlarged liver and spleen underscores the tissue remodeling in these infections. It is not known how *Leishmania*-derived molecules contribute to this disease presentation. To address this knowledge gap, we hypothesized that extracellular vesicles (EVs) released from *Leishmania*-infected cells (LiEVs) that contain parasite derived molecules, induce cellular responses that promote tissue remodeling. Infection in animals was tracked by changes in liver weight and by histochemical analyses of infected livers. To evaluate the responses of macrophages *in vitro,* peritoneal exudate cell (PEC) responses to LiEVs was obtained by RT-PCR amplification of M1 targets (iNOS, IFNγ and TNFα) and M2 targets (Arginase 1 (Arg-1), IL-10 and IL-4R). For *in vivo* analysis, F4/80+ cells were monitored by immunohistochemical staining for markers of M1 or M2 polarization subsets. After overnight incubation of PECs, EVs prepared from Salmonella-infected RAW264.7 macrophages or LPS treatment induced robust expression of the M1 markers iNOS, IFNγ or TNFα. In contrast, LiEVs induced the expression of Arg-1, IL-10, and IL-4. The Salmonella derived EVs did not induce expression of M2 targets in PECs. Next, *in vivo* studies revealed a 3- and 4-fold increase of F4/80+ cells in *L. donovani*-infected livers at 20- and 42-days Post Infection (PI), respectively, as compared to uninfected livers. Immunohistochemical analysis showed that IL-4R expression by F4/80+ cells in infected livers was widespread as compared to iNOS that was expressed more discretely by cells within granulomas. The *in vitro* studies suggested that LiEVs released from

Leishmania-infected cells have the capacity to induce macrophage polarization to the M2 type. The *in vivo* studies that showed more widespread IL4R expression of macrophages in infected tissue, provided strong evidence of M2 polarization in *L. donovani* infected tissues, which would ensure parasite persistence.

Keywords CYTOKINES; IL-4; IL-10; *Leishmania donovani*

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O10-04: EFFECT OF EXTRACELLULAR VESICLES SHEED BY *Leishmania shawi* ON IMMUNOMODULATION OF MURINE MACROPHAGES

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Leishmaniasis is a parasitosis caused by different species of the genus *Leishmania* and transmitted through the bite of several species of sandflies. Depending on the infecting species and the host immune competency, this disease can present different clinical manifestations: cutaneous, mucocutaneous and visceral. The treatment of this infection is time-consuming and is associated with a considerable number of severe side effects, not having many therapeutic alternatives available. *L. shawi* is one of the species that cause cutaneous leishmaniasis in humans in the South America. Macrophages (MØ) are phagocytic and antigen-presenting cells of the innate immune defense system, being also the target cells for *Leishmania* parasites. Therefore, understanding how the interaction between the parasite and its host takes place may reveal new opportunities for the prevention of the infection and/or the development of therapeutic tools. An element that has been considered key in this host-parasite interaction is the extracellular vesicles (EVs). This vesicles are produced by all type of cells and contain protein, lipids and acid nucleics and constitute an ancestral

system of cell-to-cell communication. *Leishmania* promastigotes naturally produce EVs by parasites during their life cycle. Thus, this study aimed toevaluate the immunomodulatory potential of EVs shed by *L.shawi* promastigotes on MØ activity. Therefore, murine macrophagic cell line P388D1 was used as cellular model and exposed to EVs isolated from *L. shawi* axenic promastigote culture. EVs incorporation by cells was followed by fluorescence microscopy. Immune activaction status of the cells was assessed by quantifying nitric oxide (NO) and urea production by colorimetric assays, and examining the expression of class I (MHCI) and class II (MHCII) molecules of major histocompatibility complex by flow cytometry. Cytokine and innate immune receptors gene expression was determined by semi-quantitative real-time PCR. *L. shawi* EVs were fast incorporated into MØ, promoting NO production and reducing urea release, generating an unfavorable environment for the intracellular survival of the parasite. Furthermore, EVs increased $MHCI^{+}$MØ and $MHCII^{+}$MØ population and modulated cytokine gene expression, mainly generating IL-1β and IL-12p40, showing that these cells can present antigens to T lymphocytes, establishing a bridge between innate and acquired immune response. The expression of innate immunity receptors NODs and TLRs was also modulated by these EVs. Thus, *L. shawi* EVs seem to have potential as possible study targets for future development of anti-*Leishmania* prophylactic or therapeutic products.

Keywords *Leishmania shawi*; CUTANEOUS LEISHMANIASIS; EXTRACELLULAR VESICLES; IMMUNOMODULATION

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O10-05: MICRORNAs TARGET ADHERENS JUNCTION PATHWAY IN CUTANEOUS LEISHMANIASIS

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American Tegumentary Leishmaniasis (ATL) is an endemic disease in Latin America being mostly caused in Brazil by *Leishmania (Viannia) braziliensis*. Clinical manifestations vary from mild, localized cutaneous to an aggressive mucosal form, and the host immune response strongly determines the outcome of the infection and the lesion development. However, the mechanisms leading to the progression or healing of the lesions and ulcer formation are still not well known and microRNAs (miRNAs) may be involved in the pathogenesis of CL. Considering the poor understanding of the mechanism that causes ulcer formation and the lack of evidence on the involvement of miRNAs in the pathogenesis of ATL, where inflammation has an important role, the aim of the present study was to evaluate the miRNA expression in ATL. We searched in plasma of patients infected with *L. (V.) braziliensis* with active cutaneous lesion, self-cured, healed without any specific treatment, and healthy controls, and in parallel, the miRNA expression in *L. (V.) braziliensis* promastigote-infected human monocytic THP-1 cells *in vitro*. To carry out the present study cDNAs were synthesized with Kit miScript II RT Kit (Qiagen) from total RNA both from *in vitro*

experiment and from patients plasma. Then they were submitted to qPCR array to analyze expression of 84 microRNAs associated to autoimmune and inflammatory response using the kit miScript microRNA PCR array (QIAGEN). We identified 14 differentially expressed miRNAs in self-healed individuals, eight of them up-regulated and six down-regulated in relation to the control. When comparing self-healed patients with the active disease group, we found 23 significantly different miRNAs 14 of these up-regulated and nine down-regulated. Considering all differentially expressed miRNAs, we searched for target genes and metabolic pathways using Diana MiRPath 3.0 platform as the chosen algorithm to predict miRNA/mRNA interactions. Two of these miRNAs (hsa-miR-15b-5p, hsa-miR-29b-3p) were related to the pathway of adherens junction with 28 possible target genes. Our data suggest the involvement of miRNAs in the development of the lesion in patients with ATL likely modulating the gene translation related to the adherens junction pathway.

Keywords *Leishmania (Viannia) braziliensis*; microRNA; TEGUMENTARY LEISHMANIASIS; SELF HEALING; THP-1 CELLS; PATHOGENESIS

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O17-01: MUCOSAL INNATE SUBSETS TARGETED BY *Leishmania braziliensis* DISSEMINATED FROM PRIMARY SKIN INFECTION IN A MURINE MODEL

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Mucosal leishmaniasis (ML) is a debilitating disease caused by *Leishmania braziliensis* (*Lb*) with a high potential for producing highly disfiguring scars. The factors involved in ML progression and parasite colonization of the mucosa are still not understood. *L. braziliensis* is the main causative agent of American cutaneous leishmaniasis (ACL), characterized by ulcerated skin lesions that develop at the infection site. It has been reported that 1-20% of ACL cases progress to destruction of the mucosa of the tongue, nose and throat years or decades after apparent clinical cure of primary lesions. Despite the impact caused by ML in South America, there is insufficient research done on this disease and a complete lack of experimental models. Here, using immunodeficient mice lacking T and B cells (RAG1$^{-/-}$) and *Lb* isolated from human mucosa (*Lb*-ML), we detected parasite migration to the tongue of mice initially infected intradermally on the ear pinnae. At 16 weeks post-intradermal (i.d.) injection of 10^5 metacyclics *Lb*-ML engineered to express red fluorescent protein, parasites were detected in the tongue of infected RAG1$^{-/-}$ mice by flow cytometry and limiting dilution assay, with 10^5 and 10^4 parasites colonizing the ear and the tongue, respectively. Splenomegaly onset between 12-16 weeks post-infection (~2 fold change) positively correlated with *Lb*ML migration to the tongue, suggesting a systemic response may be involved in the dissemination. Concurrent visceralizing *L. infantum* (*Li*-VL) and self-limiting cutaneous *L. major* (*Lm*C-

CL) infections did not disseminate to the tongue of control RAG1$^{-/-}$ mice. Flow cytometry analyses of innate myeloid cells in *Lb*-ML-infected versus uninfected mice revealed an increased number of neutrophils and inflammatory monocytes in the ear, while in the tongue a consistent decrease in dendritic cells and an increase in F4/80^{hi} macrophages were indicative of infection. Single cell RNA-seq (scRNA-seq) analysis of CD45^{+} sorted cells from infected tongues at 16 weeks post-infection revealed significant changes in the mononuclear phagocyte subsets when compared to uninfected tongues, based on their transcriptomic profiles. An enriched population of macrophages expressing interferon-inducible GTPases in the infected tongue was the major cluster carrying *Lb*-derived sequences. Also in this transcriptomic analysis, interferon-gamma was upregulated in NK cells from infected samples. Taken together, this work represents a novel investigation of the host oral mucosa immune landscape during *Lb* dissemination from skin and suggests an inflammatory milieu in the oral mucosa despite absence of pathology in RAG1$^{-/-}$ mice. Ultimately, this experimental model may provide a way forward to study pathways involved in *Lb* dissemination inside the host and evaluate candidate drugs to pharmacologically target them.

Keywords ORAL MUCOSA; *Leishmania braziliensis;* DISSEMINATION; SCRNA-SEQ; TRANSCRIPTOMICS

O17-02: ALTERED MONOCYTE RECRUITMENT AND ACTIVATION FOLLOWING CHALLENGE INFECTION WITH *Leishmania amazonensis* ABROGATES PROTECTIVE TH1 IMMUNITY

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Despite the development of numerous experimental vaccines that mediate protective immunity against cutaneous and visceral forms of experimental murine leishmaniasis, "leishmanization", the inoculation of viable *Leishmania major* (*L.m.)* parasites into the skin, remains the only 'immunization' strategy known to generate protection against subsequent natural sand fly-vector transmission in humans. In this setting, rapid recruitment of $CD4^+$ T helper 1 (Th1) effector cells and the rapid activation of skin-infiltrating monocytes generates a hostile environment for parasite replication. Heterologous protection mediated by leishmanization with *L.m.* has been reported against experimental challenge infection with *L. infantum* and *L. donovani*. *Leishmania amazonensis (L.a.)* infection represents an important public health problem in South America and is associated with all three clinical forms of human leishmaniasis: cutaneous, muco-cutaneous and visceral. The aim of our study is to understand if concomitant immunity provided by primary *L.m.* infection can mediate protective immunity against non-healing *L.a.* challenge in the C57BL/6 mouse model of intra-dermal (i.d.) ear challenge infection. Mice were inoculated with 10^4 *L.m.* or *L.a.* parasites into the sub-cutaneous (s.c.) space of the footpad, and after healing (*L.m.)* or stabilization (*L.a.*) of the primary f.p. infection, were challenged

with 2x10^5 *L. amazonensis* or *L. major* into the ears i.d.. Lesion development and parasite load was followed up to 20 weeks post-secondary challenge infection. We characterized the recruitment and activation phenotype of different innate and adaptive immune cells. We found primary *L.a.* infection provided almost no protection against homologous secondary challenge with *L.a.* and that while *L.m.*-mediated concomitant immunity provided complete protection against homologous *L.m.* challenge, it provided only transient protection against heterologous *L.a.* challenge for the first 6 weeks post-challenge, followed by a slow but progressive increase in parasite load and lesion size. The lack of protection seen after *L.a.* challenge occurred despite equally robust early Th1 immunity, characterized by increased levels of IFN-g, TNF, STAT1, T-bet and iNOS expression at the lesion site. Employing STAT6$^{-/-}$, IL-10$^{-/-}$, and STAT6$^{-/-}$IL-10$^{-/-}$ (dKO) mice we observed that both STAT6 and IL-10 play a partial role in the lack of protection after *L.a.* challenge. Different from *L.m.* challenge, *L.a.* challenge induced continuous monocyte recruitment to the site of infection and these cells acquired not only iNOS but also arginase I expression. In addition, *L.a.* infection altered monocyte maturation at the challenge site when compared to *L. major* infection. Following co-culture with apoptotic *L. amazonensis* infected neutrophils *in vitro*, infected monocytes preferentially matured towards a macrophage, over a dendritic cell phenotype, characterize by a delay in both decreased CCR2 expression and acquisition of CD64 expression. In conclusion, manipulation of monocyte maturation by *L.a.* parasites, in addition to the continuous recruitment of permissive immature CCR2$^+$ monocytes to the site of infection, can overcome otherwise protective Th1 immunity. These data suggest that novel strategies for immunization might be necessary against *L.a.* parasites, combining the generation of Th1 immunity while also balancing the recruitment and maturation of permissive cells at the site of infection.

Keywords*Leishmania;* MONOCYTE; TH1; RECRUITMENT, MATURATION

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O17-03: CHARACTERIZATION OF MACROPHAGE ACTIVATION AFTER EXPOSURE TO PLASMA FROM PATIENTS WITH CUTANEOUS LEISHMANIASIS AND ITS RELATIONSHIP WITH PATHOGENESIS

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The macrophage (MØ), the main host cell of *Leishmania*, is characterized by its phenotypic and functional plasticity which allows participation both in pathology and in the protective and curative response to infections. Different polarizing signals are found in blood circulation, which modulate the expression of characteristic genes of each subtype. Antibodies act as activators of the immunosuppressive phenotype of the MØ (M2). In patients with severe forms of leishmaniasis, high titers of specific antibodies against the parasite have been found and, in turn, it has been shown that M2 is associated with parasite survival, chronicity and severity of the disease. In addition, it has been reported that exposure to antileishmanial drugs repolarizes the M2 phenotype to the proinflammatory phenotype (M1) in successful treatments. Here, we evaluated the activation profile of monocyte-derived MØ after exposure to plasma from asymptomatic individuals (n=5), patients with chronic CL (n=5) and healthy donors (n=2), in order to establish whether components of human plasma participate in differential macrophage activation and potentially in disease susceptibility. U937 cells were exposed for 7 days to RPMI medium containing human plasma. RNA sequencing revealed 205 genes differentially expressed between macrophages stimulated with plasma from asymptomatic and chronic CL patients. Among enriched pathways from genes over-expressed in monocytes exposed to plasma from chronic patients (in contrast to asymptomatic individuals) were steroid metabolism, cholesterol biosynthesis and anti-inflammatory signaling (IL10, IL-37 and LTC4-

CYSLTR) , the latter which favor survival of intracellular *Leishmania*. Among downregulated were those related to extracellular matrix organization. Given the stimulation of asymptomatic plasma, the overexpressed genes were associated with activation of the inflammasome, recruitment of proinflammatory cells and purinergic signaling that has been related to cell death and control of Leishmania. These results suggest that there are molecules in the plasma of patients with chronic CL that differentially activate signaling pathways compared to plasmas from healthy and asymptomatic individuals, and that these are predominantly associated with induction of an anti-inflammatory macrophage profile.

Keywords LEISHAMANIASIS; RNA-SEQ; TRANSCRIPTOME; MACROPHAGE; PHENOTYPE

O17-04: ROLE OF TLR9 IN THE EXPRESSION OF CD200 MODULATING *Leishmania amazonensis* VIRULENCE *IN VIVO*

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Leishmaniasis is a complex of devastating diseases affecting different tropical and subtropical areas by virulent protozoan species named *Leishmania*. The virulence of these parasites depends on the capacity of infective forms named amastigotes to modulate and inhibit the host immune response. Amastigotes of *Leishmania amazonensis* induce the expression of the host ligand CD200, an immunomodulatory glycoprotein, which in contact with its receptor (CD200R) inhibits iNOS/NO signaling pathways favoring intracellular survival. CD200 induction depends on the activation of TLR9/MyD88/TRIF signaling pathways by DNA-containing extracellular vesicles released by intracellular amastigotes. Here we investigate the infection process and analyze the macrophage population ($CD45^+CD11b^+F4/80^+$) recruited at the lesion in the footpads of WT and $TLR9^{-/-}$ mice by flow cytometry infected with *L. amazonensis*. As early as the third week of infection, infected $TLR9^{-/-}$ mice developed much smaller lesions, containing a reduced number of parasites recovered at the end of the experiment. As expected, WT mice showed rapid growing and non-healing lesions typical of *L. amazonensis* infection that had an increased parasite load in the infected tissue, relative to mild infection observed in $TLR9^{-/-}$ mice. When recovered from the lesions, double positive CD45 and CD200 expressing cells ($CD45^+CD200^+$) were significantly higher in WT than $TLR9^{-/-}$ mice. More importantly, a marked difference was observed in the absolute number of macrophages ($CD45^+CD11b^+F4/80^+$ cells). Finally, WT mice contained 3 folds more macrophages than $TLR9^{-/-}$ mice, and those cells expressed significantly more CD200 than the normalized number of macrophages from $TLR9^{-/-}$ mice. We showed that the impairment of CD200

expression reduces macrophage recruitment in TLR9$^{-/-}$ mice lesions. Further studies will describe the kinetic of CD200 induction and the other cells involved in controlling immune response modulated by *Leishmania* parasites.

Keywords AMASTIGOTES; CD200; *Leishmania amazonensis*; MACROPHAGES; TLR9

Financing FAPESP; CNPq; CAPES

O17-05: DIABETES MODIFIES THE CLINIC PRESENTATION OF CUTANEOUS LEISHMANIASIS

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Diabetes mellitus (DM) is a chronic disease characterized by high blood sugar levels. Currently, due to genetic characteristics, a poor eating habits and a sedentary lifestyle, about 90-95% of diabetes cases are type 2, characterized by peripheral insulin resistance. DM increases the risk of extracellular bacterial infections and fungal infections, and changes the clinical presentation and response to treatment of these infections. The cutaneous leishmaniasis (CL) is a disease that predominantly affects adults, young males, but the number of children and the elderly with the disease has increased in recent years. The study included 36 DM patients with CL and 36 patients with CL without DM aged 18 to 60 years who sought the reference center of Corte de Pedra from January 2017 to June 2020. The diagnosis of CL was performed by detection of L. braziliensis DNA in tissue biopsies. DM was diagnosed when glycated hemoglobin was ≥6,5. Mononuclear cells of the peripheral blood were stimulated with soluble leishmania antigen and cytokines were measured in supernatants by ELISA. A lesion biopsy was also performed for histopathological and immunohistochemical studies. All patients were treated with Glucantime® (Sanofi-Aventis) at a dose of 20mg/kg/day. The cure was defined by the complete healing of the ulcers on day 90 in the absence of elevated borders.

There was no difference between the 2 groups in relation to the duration of the disease, location and size of the lesion. There was no difference in the frequency of cells expressing CD20 + (lymphocyte B) and CD68 + (macrophages), however, there was a decrease in the frequency of CD8 + cells in patients with CL with DM. The most important clinical difference between the 2 groups was the presence of atypical lesions characterized by superficial ulcers without well-defined borders in 13 (36%) of the diabetic patients. Moreover, while there was no difference in the cure rate in DM + CL (67%) and in CL without DM (56%), $P>0.05$, the cure rate in diabetes with atypical lesions was 31% and in those with typical lesions was 87%, $P<0.01$. The production of IFN-γ, TNF, and IL-1β was higher in patients with atypical lesions than in patients with typical lesions. The most important finding herein was documentation of the capability of DM to modify the clinical presentation of CL, due to the appearance of atypical CL lesions associated with a poor response to therapy. In our study, the atypical lesions observed were predominantly flat, usually large, and lacked well-defined borders. While more superficial ulcers may occur due to a lower frequency of cytotoxic CD8+ T cells at the lesion site and decreased necrosis, the high rate of failure of therapy seen in these patients may be occurring due to an increase in the synthesis of proinflammatory cytokines. **Conclusions:** There was no evidence that sugar blood levels influenced the presentation and response to therapy of CL. However diabetic patients who had an exaggerated inflammatory response had atypical lesions and more failure to therapy.

Keywords CUTANEOUS LEISHMANIASIS; DIABETES MELLITUS; IMMUNE RESPONSE; LEISHMANIASIS

O17-06: IMPAIRED TH1 IMMUNE RESPONSE IS ASSOCIATED WITH DISEASE SEVERITY AND TREATMENT OUTCOME IN CUTANEOUS LEISHMANIASIS PATIENTS

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Control of *Leishmania* infection relies on cell-mediated immune response, as IFN-γ induced activation of macrophages is known to be the main mechanism of parasite killing within these cells. Peripheral blood mononuclear cells (PBMC) from patients with cutaneous leishmaniasis (CL) caused by *Leishmania braziliensis* infection, secrete high levels of IFN-γ and TNF that contributes for parasite control but also is associated to pathology. Moreover, CL patients presents a positive delayed-type hypersensitivity reaction to *Leishmania* antigens as known *Leishmania* skin test. Interestingly, around 4% of patients with CL present a negative LST. Here, we compare the clinical presentation, response to therapy, and immune response of CL patients who are LST negative (n =72) with those who are LST positive (n =72). Patients with negative LST had significantly larger ulcers than LST positive group. Furthermore, number of lesions was higher in LST negative subjects compared to LST positive patients. While cure at day 90 post therapy was 70% in the control group, it was 43% in the LST-negative patients. In addition, ulcers healed slower in the LST-negative patients than in the LST-positive. To evaluate immune response, cytokines production in lesion biopsy supernatants was assessed by ELISA. LST-negative patients had a poor Th1 response (IFN-γ and TNF) but levels of IL-1β, IL-6, IL-17, granzyme B, and perforin were similar to the LST-positive group. In this study, we observed that in LST-negative patients, impaired Th1 response is associated with therapeutic failure. Moreover, high production of innate related inflammatory cytokines, and cytotoxic molecules such as granzyme B and perforin contributes to immunopathology.

Keywords CUTANEOUS LEISHMANIASIS; LEISHMANIA SKIN TEST; TH1 RESPONSE; INFLAMMATION; IMMUNOPATHOLOGY

O18-01: *Leishmania amazonensis* PROMOTES LONG-TERM SURVIVAL OF HOST MACROPHAGES BY INCREASING THEIR RESISTANCE TO APOPTOSIS, PYROPTOSIS, AND NECROPTOSIS

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Leishmania parasites infect key innate immune cells such as macrophages. To promote their intracellular survival, these parasites have evolved various strategies to exploit the phenotypic plasticity and subvert key immuno-metabolic functions of their host cells. Cellular suicide through Regulated Cell Death (RCD) represents an ultimate sacrifice of host cells to limit infection. RCD occurs through defined processes that regulate the inflammatory response during infection, including anti-inflammatory apoptosis, and pro-inflammatory necroptosis and pyroptosis. As expected by the ever-evolving host/pathogen arms race, intracellular microbes exploit RCD pathways to guarantee host cell survival thereby promoting chronic infection. Even though increased macrophage survival has been previously linked to *Leishmania* infection, a systematic analysis on the impact of intracellular parasites on different host cell RDC pathways has not been conducted. To overcome this limitation, we applied phenotypic and transcriptomic analyses on *L. amazonensis*-infected, primary macrophages in the absence and presence of RCD-stimulating factors**.** C57BL/6 bone marrow-derived macrophages (BMDMs) were differentiated in presence of CSF-1 and infected with lesion-derived *L. amazonensis* amastigotes (MOI of 4 to 1). Infected BMDMs were monitored up to 50 days post-infection (PI) under normal or RDC-inducing conditions such as CSF-1 deprivation

(intrinsic apoptosis) or actinomycin D treatment (extrinsic apoptosis), LPS/ATP stimulation (pyroptosis), and z-VAD/LPS treatment (necroptosis). Cell death was monitored by YO-PRO-1 staining, and transcriptional responses induced by infection and different RCD stimuli were determined by RNA-Seq at day 3 PI, or RT-qPCR at days 0, 3, 15, and 30 PI. In presence of suboptimal concentrations of CSF1, more than 60% uninfected macrophages died by apoptosis within the first 15 days. In contrast to the majority of *L. amazonensis*-infected BMDMs survived for over 50 days PI despite the dramatic increase in parasite load of over 100 amastigotes per cell. Long-term cell survival and resistance to cell death-inducing signals correlated with highly reproducible transcriptomic signatures affecting RDC pathways, with statistically significant expression changes observed for 64.5, 76.3, and 60.3% of genes associated with apoptosis (extrinsic and intrinsic pathways), pyroptosis (inflammasome priming/activation) and necroptosis, respectively. A coordinated decrease in pro-RCD and an increase in anti-RCD gene expression revealed a dual inhibition of host RCD pathways in infected BMDMs. These expression changes included a series of RCD key regulators that are common to the different forms of cell death, including casp8, fadd, tradd, tnfaip3, tax1bp1, birc3, and itch. In conclusion, our analyses firmly document the pan-anti RCD effect of *L. amazonensis* on its macrophage host cell. We establish a first, in-depth transcriptomic profile underlying long-term host cell survival in the presence of the parasite, and show that this phenotype correlates with anti-apoptotic, -pyroptotic and -necroptotic expression patterns that are rapidly induced and maintained for at least 30 days post-infection. Our findings shed important new light on mechanisms underlying *Leishmania* chronic infection and propose these parasites as unique biological tools to investigate mammalian cell survival.

Keywords *Leishmania amazonensis*; MACROPHAGE; SURVIVAL; APOPTOSIS; PYROPTOSIS; NECROPTOSIS

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O18-02: LONG-TERM HEMATOPOIETIC STEM CELLS AS SANCTUARY NICHE DURING TREATMENT FAILURE IN VISCERAL LEISHMANIASIS

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Given the discontinuation of various first-line drugs for visceral leishmaniasis (VL), large-scale *in vivo* drug screening, immunophenotyping, transcriptomics and a systems biology approach were combined to study persistent infections and therapeutic failure. Double bioluminescent/fluorescent *Leishmania infantum* and *L. donovani* reporter lines enabled the identification of long-term hematopoietic stem cells (LT-HSC; Lin- Sca1+ cKit+ CD48- CD150+) as a hospitable cellular niche with exceptionally high parasite burdens, a feature also confirmed for human hematopoietic stem cells (hHSPC, CD45^{lo} CD34+). LT-HSC suppress nitric oxide and reactive oxygen species, are more tolerant to antileishmanial drug action as a consequence of the extreme parasite burden, and serve as source of systemic parasite spread and relapse. A unique transcriptional "*StemLeish*" signature in these cells was defined by upregulated TNF/NF-kB and RGS1/TGF-β/SMAD/SKIL signalling, and a downregulated oxidative burst. Cross-species analyses demonstrated significant overlap with human VL and HIV co-infected blood transcriptomes. Silencing of the major

differential gene *Rgs1* confirmed the importance of the *StemLeish* signature in regulating infection of LT-HSC. In summary, the identification of LT-HSC as a drug- and oxidative stress-resistant niche, undergoing a conserved transcriptional reprogramming underlying *Leishmania* persistence and treatment failure, may open new therapeutic avenues for leishmaniasis.

Keywords VISCERAL LEISHMANIASIS; BONE MARROW; HEMATOPOIETIC STEM CELLS; PERSISTER; POST-TREATMENT RELAPSE

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O18-03: IMMUNOSUPPRESSIVE TREATMENTS AFFECT THE DEVELOPMENT OF VISCERAL LEISHMANIASIS

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The recent increase in the use of immunosuppressive agents to treat autoimmune diseases in endemic areas of visceral leishmaniasis (VL), has led to a higher susceptibility to develop this severe form of the disease. Few studies have examined the immune response developed under this type of immunosuppression during *Leishmania* infection. For that reason, the objective of this work was to characterise in mice the evolution of *Leishmania infantum* infection and the related immune response after the immunosuppression with the main immunosuppressants used in clinical practice (methotrexate -MTX-, TNF antagonist – anti-TNF- and methylprednisolone –MPDN). C57BL/6 mice were immunosuppressed with such treatments at clinical doses and subsequently infected with 10^7 promastigotes of *Leishmania infantum*. Immunosuppression conditions were maintained during the four weeks of infection, after which the parasite load was assessed by quantitative PCR in the target organs (liver, spleen and bone marrow). In addition, the levels of specific IgG against the parasite were determined by ELISA. To evaluate the protective cellular immune response the expression of specific pro-inflammatory cytokines, IFN-γ, TNF and IL-2, by $TCD4^+$ and $TCD8^+$ lymphocytes was examined through flow cytometry. The results showed that immunosuppression induced by anti-TNF leaded to a greater severity of infection as it increased the parasite load on the liver, as well as specific IgG antibodies against *Leishmania infantum* with respect to the control group. In addition, this treatment reduced the production of the pro-inflammatory cytokines IFN-γ, TNF and IL-2, leading to a less effective cellular response to the parasite. However, MTX produced

a decrease in the parasite load on the spleen, probably due to its antiparasitic effect. Both MTX and MPDN generated an increase in the production of multiple pro-inflammatory cytokines, which suggest a better response against VL. In conclusion, these results show that the immunosuppressant agents studied had a different impact on the parasitic load and immune response to *Leishmania infantum* infection, being the TNF antagonist immunosuppressed group having the worst prognosis. Therefore, it would be convenient to assess the most appropriate treatments for those patients who live in endemic areas of leishmaniasis.

Keywords VISCERAL LEISHMANIASIS; IMMUNOSUPPRESSION; MICE; PARASITE LOAD; CYTOKINES

O18-04: REVISITING HOST CELL SUBSTRATES OF THE *Leishmania* METALLOPROTEASE GP63

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The interaction between *Leishmania* metacyclic promastigotes and phagocytic cells involves the action of parasite effectors including the zinc-dependent metalloprotease GP63. This virulence factor contributes to the migration of promastigotes through the extracellular matrix, the modulation of parasitophorous vacuole function, and the impairment of key signaling pathways involved in macrophage activation. The mechanism by which GP63 alters those host cell processes is associated to the cleavage of various proteins, leading to macrophage attenuation and enhancement of parasite survival. Given the abundance and powerful proteolytic activity of GP63, unambiguous identification of its substrates in host cells hinges on the adequate preparation of cell lysates to prevent artefactual post-cell lysis degradation. However, in a number of previous studies aimed at investigating the impact of GP63 on the cleavage/degradation of host cell substrates, the presence of zinc metalloprotease inhibitors in lysis buffers was not specifically mentioned. To discard the possibility that some host cell proteins were wrongly assigned as GP63 substrates due to post-cell lysis proteolytic events, we sought to re-evaluate the cleavage/degradation of previously reported GP63 substrates. To this end, we infected bone marrow-derived macrophages with either wild type, *Δgp63*, and *Δgp63+GP63 L. major* metacyclic promastigotes for various time points. We prepared cell lysates in the absence or presence of the zinc-metalloprotease inhibitor 1,10-phenantroline and examined the levels of select previously reported GP63 substrates. We show that inhibition of *L. major* GP63 activity with 1,10-phenantroline during the lysis of macrophages prevented the cleavage/degradation of several previously described GP63 targets, including PTP-PEST, mTOR, p65RelA, c-Jun, VAMP3, and NLRP3.

Conversely, we confirmed that SHP-1, Syt XI, Syntaxin-5, and VAMP8 are genuine GP63 host cell substrates. These results point to the importance of inhibiting GP63 activity during the preparation of *Leishmania*-infected host cell lysates. In addition, our results indicate that the role of GP63 in *Leishmania* pathogenesis must be re-evaluated.

Keywords Leishmania; GP63; METALLOPROTEASA; 1,10 PHENANTHROLINE; HOST-PHATOGEN INTERACTION

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O31-01: VALUE OF *Leishmania* WHOLE BLOOD STIMULATION ASSAYS IN HIV PATIENTS: FROM ASYMPTOMATIC INFECTION TO OUTCOME PREDICTION

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For over a decade, the difficulty of correctly identifying asymptomatic *Leishmania* infection or past exposure has formed a bottleneck to address transmission rates. Likewise, low-invasive and predictive biomarkers to detect the onset of visceral leishmaniasis (VL), VL relapse, or to monitor treatment efficacy are still lacking. In this work, we focus on the previously described 'whole blood cytokine-release assay (WBA)' as an easy, fast, and sensitive measure of the *Leishmania*-specific cellular immune response and promising biomarker of exposure or cure. Despite its promising results in VL patients, a comprehensive assessment of its value in HIV coinfected patients is missing. This is particularly relevant as HIV coinfected patients

experience higher rates of VL relapse and development and could thus benefit from a 'screen and treat' strategy, whereby those at the highest risk of VL would receive pre-emptive treatment. Moreover, conventional diagnostic tests showed decreased sensitivity in HIV patients. Therefore, we studied the value of WBA in the identification of asymptomatic *Leishmania* infection and to predict VL relapse over the course of 4 years in an HIV population of Northwest Ethiopia. HIV-infected patients in HIV/ART follow-up at the Abdurafi health center in Ethiopia were recruited between October 2017-2020 with 3 monthly investigations to check for asymptomatic *Leishmania donovani* infection and VL development, and VL cases were followed up to 2 years to monitor relapses. The *Leishmania* WBA was carried out in addition to a battery of conventional parasitological, molecular, and serological *Leishmania* tests on a selection of 131 HIV patients. This study included 31 long-term non-*Leishmania*-infected HIV controls, 75 long-term asymptomatically *Leishmania donovani*-infected patients, and 17 HIV patients who developed VL. Depending on the measured cytokine, the WBA was able to identify 30% more HIV patients with an asymptomatic *Leishmania* infection or past exposure as compared to several blood/urine tests (PCR, DAT, KATEX, rk39 RDT, rK39 ELISA). In addition, the levels of expression remained more stable over 2 years of follow-up compared to conventional markers. VL developers were divided into a patient group with long-term cure (cVL-HIV, n=7) and a patient group with frequent relapse (rVL-HIV, n=10). The cVL-HIV group showed an increase in IFN-γ and IP-10 levels up to 3-fold and 6-fold after one week of treatment (W1) and at the end of treatment (EOT), respectively. Likewise, we previously observed a 5- to 10-fold increase of these analytes in successfully treated immunocompetent VL patients of the same study area at W1 and EOT. Meanwhile, only an increase of 0.9-fold at W1 and 2-fold at EOT was observed in the rVL-HIV group. Together, these data indicate a detectable restoration of cell-mediated immunity against VL in both immunocompetent and immunocompromised patients, which could be used to monitor treatment efficacy and predict VL relapse, resulting in improved HIV patient management. Furthermore, the WBA could contribute to population screening for the detection of asymptomatic *Leishmania donovani* infection, past exposure, and disease progression in HIV patients in endemic areas.

Keywords ASYMPTOMATIC *LEISHMANIA* INFECTION; HIV; VISCERAL LEISHMANIASIS; MONITORING TREATMENT; BIOMARKERS

Financing Department of Economy, Science, and Innovation in Flanders (ITM-DGDC) and ISCIII (PI18CIII/00029)

O31-02: INCREASED AMPHIREGULIN EXPRESSION BY CD4⁺ T CELLS MARKS INDIVIDUALS WITH ASYMPTOMATIC *Leishmania donovani* INFECTION

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There is an urgent need to be able to identify individuals with asymptomatic *Leishmania donovani* infection so their risk of progressing to VL and then transmitting parasites can be managed. This study examined transcriptional markers expressed by peripheral blood CD4⁺ T cells that could distinguish asymptomatic individuals from endemic controls and visceral leishmaniasis (VL) patients. CD4⁺ T cells were isolated from individuals with asymptomatic *Leishmania donovani* infection, endemic controls and VL patients. RNA was extracted and RNAseq was employed to identify differentially expressed genes. Amphiregulin (AREG) was identified as a distinguishing marker of CD4⁺ T cells from individuals with asymptomatic *L. donovani* infection, compared to VL patients and healthy endemic control individuals while several genes with known roles in VL patients were found to be downregulated in asymptomatic individuals. Accompanying these transcriptional changes were alterations in the distribution of CD4⁺ T cells subsets, including increased frequencies of TEM, Tfh, Th1 and Treg cells in asymptomatic individuals compared to endemic controls and VL patients. However, of these CD4⁺ T cells subsets, Treg cells showed the largest differential in AREG expression between the three groups, indicating they were an important source of AREG in asymptomatic individuals. AREG levels in plasma and antigen-stimulated whole blood assay cell culture

supernatants were significantly elevated in asymptomatic individuals, compared to endemic controls and VL patients. In summary, Treg cell AREG was identified as an immunological biomarker of asymptomatic *L. donovani* infection suggesting the presence of an ongoing inflammatory response in asymptomatic individuals required controlling infection and that AREG may play an important role in preventing inflammation induced tissue damage and subsequent disease in asymptomatic individuals. We also established that elevated AREG protein levels could be used as a biomarker to identify asymptomatic *L. donovani* infection.

Keywords VISCERAL LEISHMANIASIS; *Leishmania donovani*; $CD4^+$ T CELL; REGULATORY T CELL; AMPHIREGULIN

031-03: IMMUNOLOGICAL DETERMINANTS OF RECURRENT VISCERAL LEISHMANIASIS IN HIV-COINFECTED PATIENTS

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Despite apparent parasitological and virological suppression, the majority of HIV-coinfected visceral leishmaniasis (VL) patients exhibit a disease course characterized by frequent VL relapse. Currently, only a few immunological determinants of recurrent VL relapses, such as a persistently low $CD4^+$ T cell count and elevated $CD8^+$ T cell exhaustion, have been

identified. However, their temporal course and causal role remain elusive. Thus, we characterized the compositional and functional changes of blood cellular immune subsets during the VL-HIV disease course in a selected group of the PreLeisH cohort in North-West Ethiopia. This unique cohort of >500 HIV patients was monitored over time to capture the progression towards (primary) active VL disease and eventual relapse or cure. To characterize the baseline host immune state during active VL-HIV disease, cross-sectional analyses were performed on: 21 HIV patients that developed VL between 2017 and 2019 (VL-HIV), 19 HIV-positive-only patients with no prior VL history, and 20 *Leishmania*-infected HIV patients that remained asymptomatic across all study timepoints (AL-HIV). Next, to characterize the prognostic value and dynamics of immune markers associated with VL relapse in VL-HIV patients, VL developers were stratified in long-term cured patients (cVL-HIV; n=7) and relapse cases (rVL-HIV; n=14) for longitudinal analyses. All patients underwent flow cytometry profiling of blood NK & T cell subset functionality (CD107, IFNγ, and IL17) and the expression of activation, differentiation, and regulatory markers (CD57, KLRG1, LAG3, PD-1, TIM3 and TIGIT). Next, PBMCs from two representative cases of each described patient group were included for paired single-cell and T cell receptor sequencing to have a more unbiased characterization. Despite a successful $CD4^+$ T cell reconstitution at six months post-treatment only in the cVL-HIV group, $CD4^+$ T cell counts were found to be similar at times of overt disease in both the cVL-HIV and rVL-HIV groups, showing little prognostic value. Consistent with previous findings, we observed higher proportions of exhaustion marker-expressing (LAG3, PD-1, TIM3, and TIGIT) $CD4^+$ and $CD8^+$ T cells at active disease development in the entire VL-HIV group as compared to HIV and AL-HIV groups. In particular, the mean proportion of PD-1-expressing $CD4^+$ and $CD8^+$ T cells across all timepoints was lower in cVL-HIV than in rVL-HIV patients. In addition, the proportion of IFNγ-positive $CD4^+$ T cells at active disease development was significantly higher in the cVL-HIV than in the rVL-HIV group. Unbiased single-cell RNA and TCR profiling demonstrated higher immune activation and T cell expansion after treatment in the $CD4^+$ and $CD8^+$ T cells of the cVL-HIV group at both the transcriptional and clonotype level. These findings indicate that relapsed VL-HIV patients show a persistent host immune dysregulation affecting mostly the antigen-presentation and antigen-recognition axis,

while cured VL-HIV patients were able to launch a discriminative and functional protective immune response already at baseline. Concludingly, we identified key immunological determinants underlying the chronic disease course that may help identify targets for host-directed therapy and guide clinical decision-making in VL-HIV co-infection.

Keywords VL-HIV COINFECTION; VISCERAL LEISHMANIASIS; HIV; IMMUNOLOGY; SINGLE-CELL

O31-04: STANDARDISATION OF DATA WITH ANNOTATED CASE REPORT FORMS FOR USE IN VISCERAL LEISHMANIASIS CLINICAL TRIALS IN PATIENTS WITH OR WITHOUT HIV COINFECTION

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Visceral Leishmaniasis (VL) remains an important cause of morbidity and mortality in several tropical regions of the world, and affects the most vulnerable populations. The Infectious Diseases Data Observatory (IDDO) is undertaking a living systematic review (LSR) to identify drug efficacy VL trials. The LSR database is available via the IDDO website (https://www.iddo.org/tool/vl-surveyor), compiling information from 161 studies conducted in 19 countries between 1983 and 2021. Past trials have shown large heterogeneities in the methodology used to assess drug efficacy, making comparability between studies quite challenging. In collaboration with the VL research community, IDDO is committed to data re-use, facilitating individual patient data (IPD) meta-analyses to address priority research questions. IDDO conducts curation from historical datasets to standardise data into a common format to enable IPD meta-analyses. However, in order to facilitate comparison of studies, optimise data quality and maximise re-use of future datasets, dissemination of common standards should be promoted. A group of experts was convened by IDDO and the Drugs for Neglected Diseases *initiative* (DND*i*) in 2020 to develop a standard annotated Case Report Form (aCRF) for uncomplicated VL efficacy trials to optimise data quality and comparability for prospective research, aiming to disseminate a common terminology across endemic

regions. We use Clinical Data Interchange Standards Consortium (CDISC) compliant terminology. During development of the uncomplicated VL aCRF, it was recognised that with falling numbers of leishmaniasis the proportion of patients with HIV coinfection were going to have increasing importance in control and elimination of the disease. These patients do not fit the standard criteria normally required within a clinical trial. Capitalising on the work done for the uncomplicated VL aCRF, an aCRF for HIV-VL coinfection has been developed in 2022 in collaboration with the Rajendra Memorial Research Institute of Medical Sciences, an institute of the Indian Council of Medical Research, DND*i*, Médecins Sans Frontières (MSF) and other key stakeholders from academia, NGOs, WHO, pharmaceutical industry, and drug regulators. These aCRFs are the first for a neglected tropical disease to have been developed based on CDISC compliant terminology, allowing for efficient generation of clinical data with downstream impact through adherence to CDISC standards recognised by stringent national drug regulatory agencies in the United States, European Union, China and Japan. A key consideration has been the end user, to drive the development of a tool that can be adapted to the data requirements of individual trials. The aCRF development is a global project, with multi-disciplinary stakeholders from Asia, Africa, the Americas and Europe engaged in their development. User Guides for each aCRF have also been compiled with expert input. The aCRFs and User Guides are freely available under a Creative Commons Attribution (CC BY) license on the IDDO website. We will present the benefits of data standardisation and the impact this will have on the VL research community. We will also discuss the challenges associated in deciding what data to capture and how, and the need to tailor aCRFs depending of the study population.

Keywords VISCERAL LEISHMANIASIS; DATA STANDARDISATION; HIV

O31-05: FEATURES OF VISCERAL LEISHMANIASIS IN A MALNOURISHED HOST AFTER *Leishmania donovani*-INFECTED SAND FLY BITES

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Visceral leishmaniasis (VL) causes spleen and liver failure and death if left untreated. The Indian sub-continent contains major foci of VL transmitted among humans exclusively via *Leishmania donovani*-infected sand fly (SF) bites. Most infected people remain asymptomatic, but those who suffer from malnutrition, associated to impairment of immune function, are at a higher risk of developing clinical VL, elevating their morbidity and mortality rates. A malnourished (MN) animal model of VL demonstrated that dissemination of intradermally-delivered *L. donovani* to visceral organs is enhanced by malnutrition through decreased recruitment of CCR2-expressing monocytes to the lymph nodes, and impairment of lymph node barrier function. Comparatively, dissemination of *L. donovani* in well-nourished (WN) animals occurred only in SF-initiated infections, via a sustained IL-1-driven inflammatory response. The two studies implicated distinct inflammatory pathways in parasite dissemination. Since clinical VL is the outcome of SF transmission and malnutrition, we developed a model of vector-initiated VL in MN mice to better understand the innate inflammatory response that mediates *Leishmania* dissemination and assess disease pathology under more natural conditions. Experimental data were blinded and the code broken post-analysis of data. Preliminary data

comparing animals infected via needle or SF bites, showed an intense recruitment of Cd11b+ myeloid cells to bite sites of WN or MW mice at 24 hrs ($P < 0.05$), with a fold-increase of 2.26 and 2.56 in neutrophil recruitment, respectivetily, when compared to needle-infected animals. An IL-1□-driven inflammatory milieu was observed at 24 hrs in both SF infected MN and WN animals, but only the MN animals sustained the response up to 72 hrs post-bites, with a 1.25-fold increase of Cd11b+IL-1□+ cells compared to WN animals ($P < 0.05$). At this timepoint, neutrophils are the major source of IL-1□, which correlated with enhanced parasite dissemination to the spleen. Importantly, neither WN nor MN animals infected via needle showed significant upregulation of cells expressing IL-1□. Preliminary qPCR data shows that parasites disseminated to the spleen in 66% of MN and 33% of WN SF-infected mice compared to none in needle-infected groups. To investigate possible impairment of cell migration in MN animals, we analyzed skin monocytes and dendritic cells expressing CCR2 and CCR7, respectively. Compared to needle-initiated infections, a significant upregulation of monocytes expressing CCR2 ($P < 0.05$) was observed at the bite site in both MN and WN animals at 24-72 hrs, reinforcing their efficient recruitment from peripheral blood to the skin. Interestingly, upregulation of CCR7, required for entry of dendritic cells into lymphatics, increased only in WN animals at 72 hrs post-bites. Over a period of 21-weeks, SF-infected MN mice exhibited clinical symptoms with 80% of animals reaching the study endpoint compared to 10% of the MN needle-infected animals ($P = 0.0010$). Animals under WN conditions did not show any VL clinical symptoms. Our early results indicate that MN mice show a distinct exacerbated VL pathogenicity. Understanding how an immune system compromised by malnutrition responds to *L. donovani* following natural transmission via a SF bite will contribute to improving VL treatment and prevention.

Keywords: *Leishmania donovani*; SAND FLY; INNATE IMMUNE RESPONSE; MALNUTRITION; ANIMAL MODEL

O31-06: BIOMARKERS OF DISEASE SEVERITY IN PATIENTS WITH VISCERAL LEISHMANIASIS CO-INFECTED WITH HIV/AIDS

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Visceral leishmaniasis (VL) is one of the deadliest tropical infection diseases, yet the coinfection with HIV virus drastically increases relapses, treatment failure and mortality. The concomitant action of these two pathogens leads to high cellular activation independently of the progression to AIDS. In addition, microbial translocation and bacterial infections are thought to contribute worsening the clinical picture. Identifying biomarkers associated with disease severity is of interest for clinical management of patients with VL-HIV/AIDS. Thus, we analyzed in the sera several markers including interleukins (IL-1β, IL-6, IL-8, and IL-17), interferon-γ (IFN- γ), tumor necrosis factor (TNF), lipopolysaccharide (LPS), soluble CD14 (sCD14), macrophage migration inhibitory factor (MIF) and intestinal fatty acid-binding protein (IFABP). These markers were compared with disease severity in 24 patients with VL/HIV presenting different clinical outcomes. Disease severity was defined by the probability of death calculated using a score set system derived by the Kala-Cal® software. Probability of death ranged from 3.7% to 97.9%, with median of 28.8%. Five patients died (20%). At the univariate analysis, disease severity was correlated with TNF, IFN-γ and sCD14. LPS was positively correlated with sCD14 specifically in patients with low $CD4^+$ count ($CD4^+$ T-cell <200 cells/mL). Most importantly, the multivariate analysis including LPS, $CD4^+$count and sCD14

showed that sCD14 was the only independent predictor for disease severity and death. The associations between theses biomarkers and the clinical presentations provide insights into the pathogenicity of lethal VL/HIV coinfection. Furthermore, our results indicated that sCD14 is a powerful marker of pathogenicity and death for patients with VL-HIV/AIDS.

Keywords BIOMARKERS; VISCERAL LEISHMANIASIS; HIV/AIDS; SOLUBLE CD14; DISEASE SEVERITY

O35-01: CONCOMITANT IL-4 AND IFN-γ CYTOKINE STIMULI INCREASE L-ARGININE PATHWAY-RELATED GENE EXPRESSION WITH MARGINAL EFFECT ON *Leishmania infantum* GROWTH

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Macrophages modulate their phenotype in response to environmental changes. In *Leishmania* infection, they are a crucial mediator of immune response determining the infection outcome. We have shown that insulin-like growth factor I (IGF-I) mRNA and protein expression are modulated by the cytokines IL-4, IL-13, and IFN-γ in macrophages infected by dermotropic species *Leishmania (L.) major*. Extrinsic IGF-I favours parasite proliferation and interfers with arginase activity and nitric oxide production during infection development. IL-4 and IGF-I are both involved in *Leishmania* intracellular proliferation acting on the L-arginine metabolic pathway and polyamines production. Here we studied the L-arginine metabolism during *L. (L.) infantum* infection, aetiological agent of potentially lethal visceral leishmaniasis. Macrophages were differentiated from THP-1 monocytes with 20ng/mL phorbol-myristrate acetate (PMA) for 24 hours, and then maintained for 48 hours in RPMI 1640 medium with 2% heat-inactivated foetal bovine serum, at 37°C and 5% CO_2 until the infection with *L. (L.) infantum* stationary phase promastigotes (MOI 8/1). In some experiments, IL-4 (20ng/mL) and/or IFN-γ (50ng/mL) stimuli were used. Parasitism was evaluated by light microscopy, relative quantification of *Igf-I*, *Arg1*, *Nos2 and Leish-Arg* mRNA by Real Time PCR, arginase activity by Urea production and

Nitric oxide by Griess reaction. The parasitism showed slight tendency to increase upon IL-4, to decrease upon IFN-γ and to increase upon concomitant IL-4 and IFN-γ stimuli after 24, 48 and 72 hours. Arginase activity increased upon IL-4, decrease upon IFN-γ, and increase with concomitant IL-4 and IFN-γ stimuli at 48 and 72 hours. The production of Nitric oxide increased only upon IL-4 and IFN-γ concomitant stimuli at 48, and 72 hours. *Arg1* and *Nos2* mRNA expression increased upon IL-4 and IFN-γ alone or used concomitantly. *Igf-I* mRNA expression increased upon IL-4, decreased upon IFN-γ and increased upon concomitant IL-4 and IFN-γ stimuli. The *Leishmania* arginase mRNA expression decreased under all cytokine stimuli. Knowing that IL-4 and IFN-γ are present during active disease, the present findings showing IL-4 and IFN-γ with marginal effect on parasitism reinforce the vain role of these cytokines. They induced an increase in *Arg1* and *Nos2* mRNA expression and *Igf-I* mRNA increased upon concomitant IL-4 and IFN-γ stimuli but with marginal effect on parasitism. The present results suggest that concomitant effect of these cytokines increase the expression of main genes involved in L-arginine metabolism and polyamine production during the infection but *L. (L.) infantum* growth is not affected by Nitric oxide, differing from what observed in infections by dermotropic *Leishmania* species.

Keywords *Leishmania infantum*; L-ARGININE; MACROPHAGES; IGF-I

Financing CAPES, FAPESP, CNPq, FINEP, LIM-38

O35-02: DIVERSE IL-4 AND IFN-γ EFFECT ON THE L-ARGININE METABOLIC PATHWAY IN *Leishmania infantum*-infected MURINE MACROPHAGE

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Macrophages are key cells in the establishment of *Leishmania* infection and depending on how they are activated, it results in progression or cure of the disease. One of the important metabolic pathways in macrophages for parasite control or growth is the L-arginine pathway that leads to the formation of nitric oxide or polyamines. In leishmaniases, the adaptive immune response is well established in the *L. major* mouse model, where Th1 and Th2 cytokines are related, respectively, to resistance and susceptibility to infection and are related to the metabolism of L-arginine. In visceral leishmaniasis caused by *L. infantum*, a different profile is observed with a mixture of Th1 and Th2 cytokines present during infection. The aim of the study was to evaluate the influence of IL-4 and IFN-γ in the modulation of the metabolic pathway of L-arginine in the *L. infantum-infected* BALB/c mouse macrophages. BALB/c mouse bone marrow-derived macrophages ($5x10^5$ cells) were infected with stationary phase *L. infantum* promastigotes (10 parasites/cell) and treated with IL-4 (2ng/mL) and IFN-γ (200U/mL) for 48 hours. Parasitism was evaluated by optical microscopy, nitric oxide was evaluated by the Griess method, and arginase activity by urea production. In the evaluation of parasitism, 82 parasites/100 cells were observed in the control group, that decreased to 46 parasites when cells were treated with IFN-γ, increased to 97 parasites when stimulated with IL-4 and decreased to 31 when stimulated with IL-4 and IFN-γ simultaneously. Evaluating the production of nitric oxide, we observed a

decrease in the production upon IL-4 and an increase upon IFN-γ, and an increase upon IL-4 and IFN- simultaneous stimuli compared with the control. In the evaluation of arginase activity, all infected groups showed an increase in the urea production, mainly under stimulation of IL-4 and IFN-γ simultaneously. In conclusion the results suggest that IL-4 does not promote *L. infantum* proliferation, on the contrary it seemingly potentiate the effect of IFN-γ on *L. infantum* growth even in the presence of increased arginase activity. We here highlight the differences in the metabolism of L-arginine when compared with dermotropic species, suggesting a distinct effect of cytokines in the *L. infantum*-infected mouse model.

Keywords CYTOKINES; VISCERAL LEISHMANIASIS; L-ARGININE; PARASITISM

Financing CAPES, CNPq, LIM-38

O35-03: ARGINASE 1 INDUCED ALTERATIONS IN THE T CELL AND MYELOID CELL COMPARTMENT ACCOUNT FOR DISEASE CHRONICITY DURING *Leishmania mexicana* INFECTION

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Control of *Leishmania* (*L.*) parasites require IFNγ-dependent type 2 nitric oxide synthase (NOS2), an enzyme that converts L-arginine into citrulline and nitric oxide (NO). NOS2 activity is counteracted by arginase (Arg) 1 and 2, both of which are induced by Th2 cytokines and cleave L-arginine into urea and ornithine, a precursor of polyamines necessary for cell proliferation. Recently, we observed that the expression of Arg1 and Arg2 steadily increased in *L. mexicana*-infected BALB/c and C57BL/6 mice during disease manifestation. Here, we studied the functional role of host cell arginases using tissue specific conditional Arg1- and/or Arg2-deficient mice during *Leishmania mexicana* induced chronic cutaneous leishmaniasis (CL). Upon *L. mexicana* infection, wild type (WT, $Arg1^{fl/fl}$) littermate mice developed non-healing chronic CL whereas $Arg2^{-/-}$ mice exhibited delayed onset of disease. In contrast, deletion of Arg1 in hematopoietic and endothelial cells ($Arg1^{\Delta Tek}$) and $Arg1^{\Delta Tek}Arg2^{-/-}$ double deficient mice showed strongly reduced pathology and ultimately resolved their skin

lesions despite parasite persistence. A similar phenotype was observed in mice deficient for Arg1 in monocytes and macrophages ($Arg1^{\Delta Cx3cr1}$), suggesting that myeloid Arg1 accounts for chronic CL. Using mice lacking IL-10 in CD4+ T cells ($Il10^{\Delta Cd4}$), IL-10 was identified as factor inducing Arg1 during infection. Moreover, liquid chromatography/mass spectrometry-based metabolomic analyses suggested that high amounts of Arg1 led to a depletion of L-arginine and a significant rise in polyamines in the infected WT skin and draining lymph node tissue. To unravel the mechanism of Arg1-induced pathology, we analyzed the infected WT and Arg1-deficient mice at 40 days p.i. when Arg1 mRNA was already upregulated in WT mice, but disease manifestation, parasite burden and the metabolic profile were still comparable between WT and Arg1-deficient mice. First, we checked whether increased Arg1 expression impeded NOS2 activity. Surprisingly, similar levels of NO were detected in WT and Arg1-deficient mice, although the expression of NOS2 protein in the infected skin was much higher in WT mice compared to Arg1-deficient mice. Despite comparable skin lesion sizes in both mouse groups, recruitment of myeloid cells to the infected skin was enhanced in Arg1 WT mice. To characterize further the phenotype of these cells, single-cell RNA sequencing (scRNASeq) of viable skin lesion cells on day 40 p.i. was performed. ScRNASeq analysis revealed distinct WT enriched myeloid subpopulations including a prominent Arg1/NOS2 double positive cluster of inflammatory macrophages and pointed to an altered differentiation of recruited monocytes in the presence of Arg1. Furthermore, Arg1-dependent micromilieu changes in skin and draining lymph node modulated the T cell compartment and led to the generation of a specific IL-10-independent T cell cluster that was associated with changes in the myeloid compartment. Together, we propose that Arg1-dependent T cell-mediated signals change the recruitment and the differentiation of monocytes in the skin of *L. mexicana*-infected mice. This leads to an enhanced pro-inflammatory immunopathology and provides cellular niches that favor parasite replication due to Arg1/NOS2 substrate competition.

Keywords *Leishmania*; ARGINASE; NOS2; CHRONICITY OF INFECTION; RESOLUTION; MYELOID CELLS

O35-05: CORRELATIONS BETWEEN INFLAMMATORY CELLS INFILTRATING IN *Leishmania braziliensis* ULCERS, SKIN TEST, AND THERAPEUTIC RESPONSE

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Human infection by *L. braziliensis* (Lb) causes a cutaneous ulcer. Tiisue obtained from the edge of these ulcers reveal the presence of a chronic inflammatory infiltrate, sometimes with granuloma formation. This infiltrate is predominantly composed of macrophages, T and B lymphocytes and plasma cells. The presence of amastigotes in the tissue associated with Lb PCR positivity is essential for the diagnosis. Lb ulcer is most often associated with positivity for the delayed hypersensitivity skin test to Lb Ag (SLT). Our research group highlighted that SLT negative patients have more antimony therapy failure. The histopathological analysis and cellular infiltrates seen in biopsies of SLT - patients revealed less inflammation, necrosis, and a higher but not siginificant number of amastigotes. Other cells and molecules expressions such as CD20, plasm cells, IL1b, granzyme, perforin, and macropahges subtypes are under analysis in an attempt to understand whether therapeutic failure depends on more specific cellular inflammatory mechanisms.

Keywords HUMAN, TISSUE, INFLAMMATION, SLT, CELLS, THERAPY

Financing INCT DT CNPq, NIH and FAPESB

35-06: PROTECTIVE CD4+ TH1 CELL-MEDIATED IMMUNITY IS RELIANT UPON EXECUTION OF EFFECTOR FUNCTION PRIOR TO THE ESTABLISHMENT OF THE PATHOGEN NICHE

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Intracellular infection with the parasite *Leishmania major* features a state of concomitant immunity in which CD4+ T helper 1 (Th1) cell-mediated immunity against reinfection coincides with a chronic but sub-clinical primary infection. In this setting, the rapidity of the Th1 response at a secondary site of challenge in the skin represents the best correlate of parasite elimination and has been associated with a reversal in *Leishmania*-mediated modulation of monocytic host cells. Remarkably, while many models of *Leishmania* control imply that Th1 immunity can eliminate parasites following infection and replication within phagocytes, the degree to which Th1 cells are reliant upon the time at which they interact with infected monocytes to mediate their protective effect has not been well defined. In the present work, we report that CXCR3-dependent recruitment of Ly6C+ Th1 effector (Th1EFF) cells is indispensable for concomitant immunity and acute (<4 days post-infection) Th1EFF cell-phagocyte interactions are critical to prevent the establishment of a permissive pathogen niche, as evidenced by altered recruitment, gene expression and functional capacity of innate and adaptive immune cells at the site of secondary challenge. While these protective Th1EFF cells may be memory cell derived, they possess a short-lived and infection-dependent Ly6C+

effector cell phenotype prior to challenge. Surprisingly, provision of *Leishmania* antigen-specific Th1EFF cells after establishment of the pathogen niche, even when Th1 cells were provided in large quantities, abrogated protection, Th1EFF cell accumulation and IFN-gamma production, and iNOS production by inflammatory monocytes. These findings indicate that protective Th1 immunity is critically dependent on activation of permissive phagocytic host cells by pre-activated Th1 cells at the time of infection and prior to the establishment of the pathogen niche. These observations imply that CD4 T cell-based vaccines against *Leishmania* should target cell populations that can execute their effector function in the skin at the time of, or within hours, of infection.

Keywords CD4 Th1 CELLS; MONOCYTES; VACCINATION; CONCOMITANT IMMUNITY; MEMORY

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O37-01: WHOLE BLOOD SAMPLES ANALYZED BY dcRT-MLPA REVEALED IMMUNE TRANSCRIPT SIGNATURES FOR PEOPLE WITH DIFFERENT CLINICAL STAGES OF CUTANEOUS LEISHMANIASIS

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In lieu of an effective human vaccine against Cutaneous Leishmaniasis (CL), it is crucial to better understand the factors that may affect the outcome of the disease, including the impact of the infection on the host's systemic immune responses. Such data may provide rational design of diagnostics and preventive measures against CL. The transcriptomic profiles of innate, adaptive and inflammatory immune-related genes in blood samples obtained from two *Leishmania tropica* endemic areas in Iran were investigated. Whole blood PAXgene samples were collected from individuals with healed CL lesions caused by *L. tropica* and patients with active *L. tropica* cutaneous lesions. Moreover, whole blood PAXgene samples were also collected from healthy volunteers in the endemic areas and after performing

leishmanin skin test (LST), these samples were categorized into asymptomatic (LST+) and healthy uninfected (LST-) groups. The results of the latter group served as the control for the system biology analyses. Altogether, quality RNA extracted from 57 blood samples were subjected to dual-color reverse-transcription multiplex ligation-dependent probe amplification (dcRT-MLPA) for profiling 144 immune-related genes. The obtained trace data were analyzed using GeneMapper software and then were normalized to *GAPDH* data as a reference gene. The normalized data were Log2-transformed for statistical analysis and the differential expression analysis was performed using R statistical computing environment. The protein-protein interaction network was analyzed and visualized by STRING. Twenty-seven differentially-expressed immune-related genes as well as distinct profiles and protein networks were identified in the studied groups. Significant changes in the expression of genes involved in multiple biological pathways were detected. Among these identified genes, a few were found as immune transcript signatures for the healed and the asymptomatic individuals. Information based on human populations that have endured or resisted the formation of CL lesions, namely the healed and the asymptomatic individuals, is gaining importance with respect to long-term immunity against CL. This study sought to compare the expressions of the immune-focused genes in the blood, including these groups of interest. Considering the documented role of interferon signaling in CL, it was not surprising that almost half of the identified genes belonged to this pathway. Although we recently reported interferon signaling as one of the main five clusters of enriched pathways in the skin lesions of ulcerative and non-ulcerative CL patients due to *L. tropica*, we observed for the first time that the components of this pathway are expressed distinctly in each studied group. Furthermore, for identification of the asymptomatic population in CL endemic areas, alternative minimally-invasive methods (instead of LST) are a priority in epidemiological and vaccine studies. In this regard, our obtained data could be considered for the development of blood biomarkers of CL asymptomaticity. Although CL lesions cause local damage to the skin, several differentially-expressed genes were observed for the first time in the blood with a potential to serve as biomarkers, particularly for the healed and the asymptomatic groups.

These results justify further explorations to identify novel blood biomarkers for different clinical stages of CL.

Keywords CUTANEOUS LEISHMANIASIS; ASYMPTOMATIC; TRANSCRIPTOMIC ANALYSES; DCRT-MLPA; BIOMARKER

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O37-02: BLOCKADE OF TLR2 AND TLR4 ATTENUATES INFLAMMATORY RESPONSE AND PARASITE LOAD IN CUTANEOUS LEISHMANIASIS

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Human cutaneous leishmaniasis (CL) caused by *Leishmania braziliensis* is characterized by a strong inflammatory response that is associated with the ulcer development. Monocytes / macrophages are the main cells that harbor the parasite and are also responsible for parasites control. Toll-like receptors signaling pathway (TLR) is the first pathogen defense systems and leads to the transcription of inflammatory mediators such as the production of IL-1β and TNF during the innate immune response. We recently showed that i*n vitro* infection with *L. braziliensis* caused CL monocytes to upregulate TLR2 and TLR4 expression, which was associated with TNF production. As TLR antagonist molecules have been used in the treatment of inflammatory diseases, our hypothesis is that the neutralization of these receptors may attenuate the strong inflammatory response observed in this disease**.** The aim of this study is to evaluate the role of TLR2 and TLR4 antagonists in the modulation of exaggerated inflammatory immune response observed in CL. Monocytes from CL patients and healthy subjects (HS) were treated with anti-TLR2 and anti-TLR4 and infected with *L.braziliensis.* The evaluation of infection and the parasite load was evaluated after cytospin preparations by optical microscopy. The expression of the oxidative burst, TNF, IL1β, IL-10, CXCL9 and CXCL10 were analyzed by flow cytometry. Cells from CL lesions were also treated with anti-TLR2 and anti-TLR4 and the evaluation of

chemokine and cytokine production by these cells was performed by enzyme-linked assay (ELISA). We observed that after neutralization of these receptors, the number of infected cells and the number of internalized parasites decreased in monocytes from CL patients. TLR2 and TLR4 neutralization also decrease oxidative burst as well IL-1β, TNF and CXCL9 production by monocytes from CL patients. Also, TNF production by cells from CL lesions decreased after TLR2 and TLR4 neutralization. The attenuation of host inflammatory response after neutralizing these receptors suggests the potential of TLR antagonists as immunomodulators in association with antimonial therapy in human cutaneous leishmaniasis.

Keywords CUTANEOUS LEISHMANIASIS; *LEISHMANIA BRAZILIENSIS*; TOLL-LIKE RECEPTORS; INNATE IMMUNITY; CYTOKINES

Financing The National Institutes Of Health (AI 136032 TO E.M.C)

O37-04: HYPOXIA PROMOTES CYTOLYTIC ACTIVITY OF CD8 T CELLS AND PATHOGENSIS IN CUTANEOUS LEISHMANIASIS

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Leishmaniasis is a disease caused by protozoan parasites of the genus *Leishmania* and the most common form of the disease is cutaneous leishmaniasis (CL). A fundamental question in CL is what regulates the development of severe disease, information that is critical to develop therapies to ameliorate disease. In a series of studies, we demonstrated CD8 T cell-dependent cytotoxicity as the main inducer of immunopathology in CL. This result was unexpected since IFN-γ production by CD8 T cells plays a protective role by promoting pathogen elimination. To resolve this paradox, we studied the CD8 T cells in different anatomic sites and found that the effector function of CD8 T cells in CL depends on their location: while CD8 T cells are cytotoxic (GzmB+) and produce little IFN-γ in leishmania lesions, CD8 T cells in the draining lymph nodes (dLN) have the opposite profile. Importantly, GzmB- CD8 T cells from dLN quickly upregulate GzmB after injection into CL lesions. By transcriptional profiling, we found that CD8 T cells in lesions and not dLN have a hypoxic signature. In vivo, we observed that leishmania lesions are hypoxic using the Oxyphor G4 oxygen probe and pimonidazole staining. In vitro, we found that induction of hypoxia was sufficient to convert GzmB- into GzmB+ CD8 T cells. In vivo, blocking the dimerization of HIF-α, a master regulator of hypoxia, with acriflavine decreased lesion development in mice. Together, our results suggest that the hypoxic microenvironment of leishmania lesions alter the function of CD8 T cells and convert protective CD8 T cells into pathogenic cytotoxic T cells.

Keywords CD8 T CELLS; IFN-γ

O37-05: PRELIMINARY STUDY ON THE EARLY IMMUNOMODULATORY ACTIVITY OF THE SECRETOME OF TWO CLINICALLY POLAR *Leishmania (Viannia) braziliensis* STRAINS

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American Tegumentary Leishmaniasis (ATL) caused by *Leishmania (Viannia) braziliensis* is associated to a spectrum of clinical forms ranging from localized self-healing lesions to multiple disseminated cutaneous lesions and mucocutaneous forms involving metastatic dissemination of parasites to the oropharyngeal mucosa. However, the mechanisms underlying the metastatic capability of this parasite are still poorly understood. The proteins secreted by *Leishmania* are critical for the establishment of the infection and are related to the subversion of the microbicidal functions of macrophages, modulating the host's immune responses and, therefore, could favor the persistence and metastatic dissemination of the parasites. In a previous *in vitro* study, we compared the immunomodulatory capability of the secretome of two *L. braziliensis* strains associated with clinically polar manifestations of ATL: one associated with localized self-healing cutaneous leishmaniasis (LCL) and one associated with disseminated cutaneous form (DL). We observed significant differences in protein abundances between LCL and DL secretomes and showed that treatment of peritoneal macrophages with LCL secretome and/or infection with LCL strain elicited the production of molecules involved in cell activation and recruitment (IL-6, CCL2, and TNF-α) at early times of stimulation/infection, whereas DL secretome/parasites did not. Such results suggested that secretomes of those strains could recruit cells differentially to the local area of infection. To test this, in the present study BALB/c mice were inoculated at the ears with the secretome of each strain,

LCL or DL, and euthanized 24h post-stimulus. The ears were collected, processed, and analyzed for the frequency of tissue macrophages, inflammatory monocytes, and neutrophils by flow cytometry. The frequency of arginase$^+$ (Arg$^+$) cells in these populations were also analyzed. Analysis of CD11b$^+$ myeloid populations, as a whole, did not show any change in these populations. However, the evaluation of subpopulations of CD11b$^+$ cells, such as neutrophils (CD11b$^+$Ly6G$^+$Ly6C^{int}) and inflammatory monocytes (CD11b$^+$Ly6G$^-$Ly6C^{hi}) showed a significant increase in animals stimulated with secretomes (p < 0.01). Furthermore, the number of inflammatory monocytes in mice that received LCL secretome were higher than in mice that received DL secretome; however, such difference was not statistically significant. In addition, we found significantly fewer (p = 0.0039) tissue macrophages (CD11b$^+$Ly6G$^-$Ly6C$^-$CD206$^+$) in mice receiving the DL secretome than in mice receiving the LCL secretome. The number of tissue macrophages in mice that received DL secretome was also lower than that observed in control animals (p = 0.0023) whereas this subpopulation in mice that received LCL secretome did not differ from the controls. The frequency of Arg$^+$ cells was similar between the groups after 24h of stimulus with the secretomes, except for Arg$^+$ neutrophils subpopulation of mice stimulated with LCL secretome, which was significantly lower than control (p = 0.0397). These preliminary results suggest that LCL and DL secretomes are able to promote recruitment of inflammatory monocytes and neutrophils, while DL secretomes seem to lead to tissue macrophage death. Additional experiments are needed to validate this data and to understand the role of the secretome of *L. braziliensis* strains in the metastatic process.

Keywords: *LEISHMANIA BRAZILIENSIS*; AMERICAN CUTANEOUS LEISHMANIASIS, SECRETOME, IMMUNOMODULATION

Funding: Inova Fiocruz Program, CNPQ-Universal, FAPERJ

O37-06: SUSCEPTIBILITY OF *Leishmania Viannia panamensis* TO MEGLUMINE ANTIMONIATE REGULATES NEUTROPHIL GENE EXPRESSION PROFILE

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Leishmania drug resistance (DR) poses a great threat to effective leishmaniasis control. DR has been generally assumed to be determined by the parasite. However, growing evidence points out that the complex interactions between parasites, host responses, and antileishmanial drugs can also contribute to DR. While neutrophils play a central role in the host response to *Leishmania* infection, their involvement in the outcome of infection with drug-resistant parasites has not been addressed until recently. Studies with laboratory-derived drug-resistant and susceptible lines of *Leishmania (Viannia) panamensis* evidenced that parasite DR altered neutrophil function. These observations were extended to neutrophils exposed to clinical strains of *L. (V.) panamensis* having different susceptibility to meglumine antimoniate (MA), a first-line antileishmanial drug. To investigate how the parasite MA susceptibility influences the host neutrophil response, we analyzed the gene signature elicited by neutrophils infected with *L. (V.) panamensis* strains of distinct susceptibility to MA. Highly purified neutrophils from three healthy donors were exposed in parallel to clinical *L. (V.) panamensis* strains of intrinsic resistance or susceptibility to MA and analyzed by RNA sequencing. For comparison, uninfected neutrophils from the same donors were similarly processed. In addition, we infected the ear dermis of BALB/c mice with parasite strains intrinsically resistant (n=2) or susceptible (n=2) to MA to assess potential differences in induction of pathology. Principal component analysis of the neutrophil transcriptome data indicated that the major source of variability

was the infection status, followed by the neutrophil donor. Interestingly, after adjustment of donor effect, the parasite susceptibility to MA was the second principal component accounting for variation. Differential expression analysis (DEA) between uninfected and infected samples identified 2473 differentially expressed genes (DEGs) in neutrophils exposed to MA-resistant parasites and 3078 DEGs in those infected with MA-susceptible parasites. Moreover, DEA between neutrophils exposed to MA-resistant parasites and neutrophils exposed to MA-susceptible parasites revealed nearly 300 DEGs. These DEGs were further analyzed by gene ontology (GO) enrichment analysis. Interestingly, genes induced by MA-resistant parasites were enriched in GO terms related to cell detoxification, while genes induced by MA-susceptible parasites were associated with inflammatory pathways. In parallel, our *in vivo* studies showed that mice infected with the MA-resistant strains developed larger lesions and had a significantly higher parasite burden than mice infected with MA-susceptible parasites. Our data showed that the neutrophil transcriptome profile changes significantly upon *L. (V.) panamensis* infection. Importantly, the neutrophil gene expression program induced by MA-resistant parasites differs from the one induced by MA-susceptible strains. Thus, the distinct transcriptional profile elicited by MA-resistant parasites might lead to an altered neutrophil activity that contributes to DR and to the increased pathology observed in mice infected with these strains. Altogether, these findings demonstrate that *Leishmania* intrinsic susceptibility to MA has a major impact on the neutrophil gene expression profile and the course of infection *in vivo*. These studies will not only provide a better understanding of the role of neutrophils in *Leishmania* DR but also potentially unveil novel mechanisms and surrogate markers to limit MA resistance and treatment failure.

Keywords DRUG RESISTANCE; NEUTROPHILS; RNA-SEQ; CLINICAL STRAINS; MEGLUMINE ANTIMONIATE

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O38-01: SEX HORMONES, CYTOKINES AND LIKELIHOOD OF DEATH IN VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) is an endemic and neglected condition in 97 countries, including Brazil, and is a public health problem in view of its geographic expansion, chronic nature, and physical, mental, and social repercussions that compromise the quality of life of the population and contribute to higher mortality indicators. It is a severe systemic inflammatory anthropozoonosis with a broad clinical and laboratory spectrum, with a high potential for letality when untreated or when associated with immunosuppressive events, such as variations in sex hormones, which can modulate the synthesis, secretion, and plasma levels of cytokines, determining the degree of exposure, susceptibility, severity, and letality indicators of the disease. Considering the need for new studies that show the action of cytokines as biomarkers and determinants of the course and clinical outcome, this study aimed to correlate the hormone levels of total testosterone and Dihydrotestosterone (DHT) with cytokines, clinical manifestations and probability of death in VL. This is a longitudinal and retrospective study carried out at a reference institution for the treatment of infectious and parasitic diseases in Teresina, Piauí, Brazil. Participated in the study 127 male patients who were admitted for diagnosis and treatment of VL between 2008 and 2020. Of these, 21 were evaluated before the start of treatment and thirty days later. Plasma levels of the cytokines IL-6, IL-8, IL-10, TNF, IL-1β, IL-12p70 and INF-γ, as well as serum

concentrations of total testosterone and DHT were considered as exposure variables. The endpoints evaluated involved the clinical conditions and the probability of death, calculated using Kala-Cal® software. Statistical analysis was expressed by descriptive and inferential measures, using the Mann-Whitney, Wilcoxon, Spearman correlation, and multiple linear regression tests to measure effects and the relationship between the variables of interest. The study was approved by the Research Ethics Committee of the Universidade Federal do Piauí and the favorable opinion was issued through protocol number 3,152,312. The participants presented a characteristic pattern of active phase disease by demonstrating high levels of inflammatory (IL-6, INF-γ) and anti-inflammatory (IL-10) cytokines. Association between the presence of vomiting with higher IFN-γ concentrations were observed. In addition, participants with epistaxis showed higher levels of IL-8. The probability of death ≥10% was correlated with higher IL-6 levels. After 30 days of treatment initiation, significant reduction in plasma levels of TNF, IL-10, IL-6, IL-8 and IFN-γ were observed. An inverse correlation of testosterone and DHT with IL-8 was found, indicating attenuation of the inflammatory response by IFN-γ signaling pathway, since this cytokine was inversely related to circulating DHT. The evidence found confirms the trend that people with VL have elevated plasma levels of inflammatory and anti-inflammatory cytokines.

Keywords VISCERAL LEISHMANIASIS; CYTOKINES; TESTOSTERONE; DIHYDROTESTOSTERONE; LETALITY

O38-02: STUDY OF CYTOTOXIC CD4+ T CELLS IN VISCERAL LEISHMANIASIS PATIENTS

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Control of Visceral leishmaniasis (VL), a parasitic disease caused by *L. donovani*, requires robust CD4+ T cells response to control the parasite replication by IFN-γ production and activation of macrophages. However, in VL patients, the anti-parasitic CD4+ T cell responses are ineffective because of unknown reasons. Our recent study on transcriptional signature of CD4+ T cell isolated from peripheral blood of active VL patients showed enhanced expression of genes related to cytotoxicity. In present study, we investigated expression of these cytotoxic molecules including granzyme B (GZMB), Granulysin, Perforin and NKG7 on different CD4+ T cell subsets and established the role of GZMB in regulating CD4+ T cells functional capacity. Peripheral Blood mononuclear cells were isolated from active and post treated VL patients and endemic controls and flow cytometry was performed. LAMP assay (CD107b expression) was performed to study the degranulation capacity of CD4+ T cells. Whole blood assay was performed to study the antigen specific cytokine production by CD4+ T cells and their association with cytotoxic molecules. Enzyme linked immunosorbent assay (ELISA) was performed to measure circulatory as well as antigen specific GZMB production. Concanamycin A (Con-A), a known v-ATPase inhibitor was used to examine the effect of GZMB inhibition on CD4+ T cells function.

We found that activated and degranulating $CD4^+$ T cells ($CD38^+CD107^+$) had higher expression of GZMB, Granulysin, perforin and NKG-7. Similarly, GZMB level in plasma and antigen stimulated whole blood culture supernatant were significantly elevated in active VL patient compared to post treatment and EC. There was no change is IFN-γ secretion when whole blood culture was stimulated with soluble leishmania antigen in presence of Con-A but a significant decline in IL-6 production was observed. In summary, VL $CD4^+$ T cells show a cytolytic phenotype and further investigations are required to understand their differentiation and function, particularly for promoting anti- parasitic immunity for host protection and effective intervention or therapy.

Keywords VISCERAL LEISHMANIASIS; $CD4^+$ T CELLS; GRANZYME; CONCANAMYCIN A; GRANULYSIN; PERFORIN

O38-06: EVALUATION OF THE HOST IMMUNE RESPONSE IN TREATED PATIENTS OF POST-KALA-AZAR DERMAL LEISHMANIASIS IN SUDAN

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Post-Kala-Azar dermal leishmaniasis (PKDL) is a dermatological complication affecting treated patients with visceral leishmaniasis (VL) caused by *Leishmania donovani*. In Sudan, up to 50-60% of those with VL will develop PKDL, with some of them possibly evolving to chronic PKDL or grade 2 and 3 severity, with disfiguring lesions over time if not treated. The current treatment regimen is unsatisfactory since it extends the use of potentially toxic drugs on patients who, on average, do not feel ill. A phase II clinical trial was implemented to assess two new combinations treatments for PKDL patients in Sudan. Arm1 consisted of a combination of Paromomycin (20 mg/kg/d) IM for 14 days and Miltefosine oral for 42 days, and Arm2 combination of AmBisome® (20 mg/kg total dose) IV over 7 days and Miltefosine oral for 28 days. A total of 110 PKDL patients were randomized in the 2 study arms (55/arm). In addition to treatment efficacy and safety, a secondary endpoint was to evaluate the host immune response before (D1), at the end (D42), and after the treatments (6M). Whole blood samples were stimulated with soluble *Leishmania* antigen (SLA) for 24 hours (WBA assay) and measured, using Luminex technology, the levels of 15 specific cytokines for Th1, Th2 and Th17 responses in SLA stimulated plasma. At D1, most of the patients showed a mixed Th1/Th2 response with a dominance of a Th1 profile, characterized by high levels of TNF-α and IFN-γ and low concentrations of IL-5, IL-13, and IL-10. After PKDL treatment (D42), the proinflammatory cytokines significantly declined while, by

contrast, the anti-inflammatory cytokines significantly increased and remained consistent throughout follow-up (6M). The results described at D1 are in agreement with the current literature in PKDL patients; however, it was unclear until now how anti-inflammatory cytokines behave shortly after treatment. Levels of IL-4 decreased during treatment which might be related with the inhibitory mechanism of action of Miltefosine. At M12, seven patients from Arm2 required rescue treatment (Arm2R). The analysis of the cytokine profile of this sub-group of patients at D1 showed that IFN-γ, TNF-α and granzyme B (GB) levels were significantly lower than those found in Arm2. After treatment (D42), the concentration of these analytes reaches similar levels to those of Arm2. But at M6, these patients presented increasing tendency of the proinflammatory cytokines, with significant reduced levels in GB. To conclude, following effective PKDL treatment, we observed a memory Th1-type response with high IFN-γ and TNF-α, which evolved to a mixed Th1/Th2 response with decreased proinflammatory cytokines at short term, and slightly recovered at 6M. Those with treatment failure expressed a different profile of cytokines before PKDL treatment and a significant decrease in GB at M6. Due to the lack of immunological samples at M12, a more specific profile associated with treatment failure cannot be described. There is a need for further research regarding the role of the developed memory response on the patient's outcome following treatment with PKDL.

Keywords PKDL; SUDAN; *Leishmania donovani*; CYTOKINES

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O40-01: CHARACTERIZATION OF THE INTERACTIONS OF B CELLS AND *Leishmania donovani*

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Polyclonal B cell activation and resulting hypergammaglobulinemia are a detrimental consequence of visceral leishmaniasis, which can be caused by the protozoan parasite *Leishmania donovani*; however, the mechanisms underlying this excessive production of non-protective antibodies are still poorly understood. To elucidate the mechanism of polyclonal B cell activation, we studied the interaction of primary splenic B cells from C57BL/6 mice with *L. donovani* amastigotes in vitro. These B cells form visible clusters with the parasite within 5 hours and show high levels of cell death within 24h hours of co-culture. Interestingly, we also observed the B cells to form long tubular protrusions when exposed to the parasite, which we characterized to be primarily actin-based with low amounts of tubulin. This is in line with literature descriptions of tunneling nanotubules (TNTs). TNTs represent a novel way of intercellular communication via the passage of material along these formed connections and have been implicated in the spread of some bacterial and viral pathogens. Indeed, we have observed parasites to be situated on these intercellular protrusions between splenic B cells exposed to *L. donovani*, suggesting that amastigotes may be gliding from one cell to another using TNTs. Given that these protrusions are induced by opsonized amastigotes, we further investigated the role of complement receptor 2 (CD21) in the induction of TNTs and found a significantly increased incidence of protrusions in B cells exposed to anti-CD21 coated latex beads. This possible role of CD21 crosslinking for the induction of membrane protrusions is further backed by the fact that we found marginal zone B cells, which differ from follicular B cells in their high expression of CD21, to be the main splenic B cell subtype undergoing protrusion formation. Interestingly, an antibody blockade of CD21 on B cells

lead to a dramatic decrease in amastigotes captured by the B cells, further pointing towards an important role of CD21 in the interaction of B cells with *L. donovani*. To elucidate a possible role of these connections in the propagation of parasite cell activation, we studied whether activation could be passed on through soluble messengers such as cytokines and found this to be insufficient to pass on the activation state between B cells. On the other hand, experiments allowing for cell-to-cell contact suggest that parasites and the activation state can be passed between B cells and even between bone marrow-derived macrophages and B cells. Taken together, we provide novel insights about the interaction of *L. donovani* and B cells and subsequent TNT formation and the possibility that this mechanism may contribute to polyclonal B cell activation and participate in the communication between macrophages and B cells in the marginal zone of the spleen during visceral leishmaniasis.

Keywords B CELLS; TUNNELING NANOTUBULES; *Leishmania donovani*; VISCERAL LEISHMANIASIS

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O40-02: A TRANSDIFFERENTIATED HUMAN MACROPHAGE-LIKE CELL LINE AS *Leishmania major* INFECTION MODEL

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Leishmaniasis is a neglected tropical disease caused by the intracellular parasite *Leishmania (L.)*, which infects macrophages as its definitive host cell. The mechanism of persistence within the human host cells remains poorly understood, partly due to limitations of genome editing methods in primary human macrophages. To circumvent this, we established a *Leishmania* infection model in BLaER1 cells, which can be transdifferentiated into a macrophage-like phenotype. Following the generation of CRISPR/Cas9-mediated knockouts in the undifferentiated stage, we can investigate the interaction between *Leishmania* and the transdifferentiated macrophage-like cells upon infection. In order to proof that the human BLaER1 cell can be used as a suitable infection model for human leishmaniasis, we first examined the immunophenotype of BLaER1 cells by flow cytometry and found it to be comparable with MCSF- or GM-CSF-derived human macrophages. Afterwards, we confirmed the susceptibility of BLaER1 for the infection with *Leishmania* promastigotes using confocal microscopy. When comparing infected BLaER1 and human macrophages, the infection rate, transformation of the promastigotes into amastigotes and cytokine responses, were very similar between these cell

types. In a second step, we used BLaER1 cells to investigate the role of the human inflammasome in the innate immune response to *L. major*. We found a stronger IL-1β response in infected wild type BLaER1 compared to infected BLaER1 with knocked out components of the NLRP3 inflammasome. Moreover, we observed no differences in infection rate between BLaER1 cells and their inflammasome knockout variants. Therefore, infection-dependent inflammasome activation in BLaER1 does not result in parasite restriction. This finding is in sharp contrast to results from murine macrophages and underlines the importance of research on *Leishmania*-mediated inflammasome activation in human cells, and the suitability of BLaER1 cells as a model to understand human macrophage immune effector functions against *Leishmania*.

Keywords *L. major*; INFLAMMASOME; CELL LINE; MACROPHAGE; HUMAN

O40-06: IDENTIFICATION OF A PULMONARY PARASITE NICHE: DO TRYPANOSOMES TAKE YOUR BREATH AWAY?

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Approximately 20% of sleeping sickness patients exhibit respiratory complications which are commonly attributed to secondary bacterial infections. The role of the parasite and immunological responses in the lung remain to be understood. Using a *Glossina morsitans* tsetse fly initiated *Trypansoma brucei* infection in mice we found that parasites rapidly and permanently colonize the lungs, representing one of the major target organs next to the adipose tissue. Trypanosomes were found by immunofluorescence staining and scanning electron microscopy to occupy the extravascular spaces surrounding the blood vessels of the alveoli and bronchi. Trypanosomes were often observed as nests of multiplying parasites exhibiting close interactions with collagen and highly active secretion of extracellular vesicles that are engaged in intercellular communication. The local immune response was analysed by flow

cytometry after 10 and 21 days of infection and was characterized by a substantial increase of $CD11b^+$ $Ly6C^+$ monocytes, $CD11b^+$ $Ly6C^+$ $F4/80^+$ macrophages and $CD11b^+$ $CD11c^+$ dendritic cells. $CD11b^+$ $Ly6G^+$ neutrophils only accumulated prominently at the late infection time point. Interestingly, parasite presence resulted in a significant reduction of $B220^+$ IgM^+ B cells, $CD11b^+$ $CD11c^{lo/-}$ $SiglecF^+$ eosinophils and TcR-β^- $NK1.1^+$ natural killer cells. Digital transcriptomics revealed infection-induced upregulation of Il-10, IFN-ɣ- and IFN-α-responses, IL-2-, IL-6- and TNF-signalling, a Th1 pro-inflammatory signature, negative immune checkpoint regulators and a predominant M1 macrophage polarization. *Il12a* and genes associated with complement and the B cell receptor were downregulated. No infection-associated pulmonary dysfunction could be detected by *in vivo* lung function measurements, mirroring the limited pulmonary complications during sleeping sickness. However, the substantial reduction of eosinophils, B cells and NK cells may render individuals more susceptible to opportunistic infections. Collectively, these observations provide essential insights in the peculiar parasite biology, immunological reactions and physiological function of a largely overlooked target organ which may trigger new diagnostic approaches for sleeping sickness.

4.6 OMICS - MOLECULAR BIOLOGY – BIOCHEMISTRY - OTHERS

O6-01: THE SKIN MICROBIOME ENHANCES TRANSCRIPTIONAL INFLAMMATORY SIGNATURES AND DELAYS CLINICAL RESOLUTION IN CUTANEOUS LEISHMANIASIS

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Cutaneous leishmaniasis (CL) caused by *Leishmania braziliensis* is associated with chronic lesions that are often difficult to drug treat. We previously found that treatment failure is associated with increased expression of cytolytic genes, including GZMB, GNLY and PRF1, as well as IL1B. Here we investigate how the skin microbiome influences host gene expression in lesions and treatment outcome. We carried out an integrative multi-omics study from 64 *L. braziliensis* patients including RNA-seq from lesion biopsies, 16 seq from skin swabs collection of bacterial isolates prior to treatment. We first assessed the total bacterial burden in lesions by qPCR of the 16S ribosomal subunit and found that patients with higher bacterial burdens exhibited delayed healing. To identify the bacteria, we performed 16S sequencing of lesion swabs and found that *Staphylococcus* was the most frequent dysbiosis observed in patients and was associated with delayed

lesion resolution. Since 50% of the *Staphylococcus* isolates, we collected were *S. aureus*, and *S. aureus* can be associated with severe infections, we asked whether lesions with high levels of *S. aureus* might be associated with inflammatory gene expression. We generated an in-house *Staphylococcus aureus* pangenome from our clinical isolates and known public references to quantify *S. aureus* transcript abundances through dual RNA-seq mapping analysis. We found that lesions with increased *S. aureus* transcripts exhibited high expression of inflammatory-related genes, such as CXCL5/8, CCL3/4, IL1A, IFNG, as wells as genes we previously reported as biomarkers for treatment failure including PRF1, GNLY, GZMB and IL1B. Together, these results suggest that the skin microbiome influences immune responses in lesions of CL patients, affecting how patients respond to therapy with antimony leading to a delay in healing. These studies suggest that antibiotics or probiotic therapies given in conjunction with anti-parasitic drugs might augment healing.

Keywords BIOMARKERS; MICROBIOME; TRANSCRIPTS; TREATMENT; FAILURE

O6-02: *Leishmania donovani*'S SECRETED ACID PHOSPHATASE: A POTENTIAL VIRULENCE FACTOR

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The protozoan parasite *Leishmania donovani* is a causative agent of the neglected tropical disease known as visceral leishmaniasis. This disease can be lethal when untreated with more than 100 000 cases annually. Studying *Leishmania* virulence factors is crucial in understanding how the parasite causes disease. One such proposed virulence factor is *L. donovani*'s most abundantly secreted protein: secreted acid phosphatase (SAcP). Since *sacp* is a multicopy and multifamily gene, traditional knockout methods have not been successful in creating an *L. donovani* knockout parasite to study SAcP's potential as a virulence factor. Here we show that using CRISPR-Cas9 technology we generated a successful knockout termed LdΔSAcP. Using whole-genome analysis we showed that *sacp* was present in three copies in wild type cells (LdWT) and that using CRISPR-Cas9 we were able to precisely delete all three copies without perturbing the genome. Since *Leishmania* are dimorphic parasites, we must investigate both life-stages: flagellated promastigotes which reside in the sand fly vector and unflagellated amastigotes which reside in the host macrophage. We showed a reduction in acid phosphatase activity in LdΔSAcP promastigotes but no defects in proliferation. Preliminary *in vitro* results further rule out a phenotypic change at the amastigote level also. Taken together these results suggest that SAcP may not be a virulence factor as was previously hypothesized although the virulence capabilities of LdΔSAcP compared to LdWT remain to be confirmed in an animal model. However, our system for studying multicopy and multifamily genes as potential virulence factors in *L. donovani* is successful and applicable to other genes. We are currently applying this method to other potential and understudied virulence factors

with the goal of gaining a better understanding of *L. donovani* virulence and disease pathology.

Keywords *Leishmania donovani*; VIRULENCE FACTORS; CIRSPR-CAS9; MOLECULAR MICROBIOLOGY; GENOMICS

O6-03: LIPIDOME OF *Leishmania (Leishmania) infantum*-INFECTED HUMAN THP-1 MACROPHAGE

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Leishmaniases are neglected topical diseases caused by parasites of the genus *Leishmania*. The most serious clinical form caused by *Leishmania (Leishmania) infantum* in the Americas is visceral leishmaniasis, which can be lethal if untreated. Besides the classic systemic manifestations of VL, changes in the plasma lipid profile are observed both in infected human and animals with an increase in triglycerides (TG), very-low density lipoproteins (VLDL) and a decrease in high density lipoproteins (HDL). In human *L. infantum* infection, we previously observed that high TG and VLDL levels increased the risk of development to active disease. In another previous study of our group, we observed in *L. infantum* promastigote-infected THP-1 macrophages changes in the expression of genes linked to lipid metabolism. Thus, changes in lipid metabolism may play a key role in metabolism of both macrophage and *Leishmania*. The aim of the present study was to characterize the lipidome of *L. infantum* amastigotes and *L infantum*-infected macrophages. In brief, THP-1 monocytic cell line was differentiated using 20 ng/mL phorbol myristate acetate for 24 hours and then maintained for 48 hours in RPMI 1640 medium with 5% heat-

inactivated fetal bovine serum, at 37˚C and 5% CO_2. THP-1 macrophage was infected with stationary phase *L. infantum* promastigotes (10 parasites/cell) for 6 hours when the experiment starts, and then it was evaluated at time zero and 24 hours post infection. At each time point, the amastigotes from the infected macrophages were recovered and purified using Ficoll 400 gradient centrifugation. Untargeted lipidomic analysis revealed an increase in several phospholipids, sphingolipids, free fatty acids and cholesteryl ester species in infected macrophages, as compared to non-infected macrophages. Interestingly, we also observed that the lipid profile of Leishmania showed a high relative percentage of free fatty acids and sphingolipids, as compared to non-infected or infected macrophages. Therefore, the present data suggest that the Leishmania-macrophage interaction led to changes in lipid profile of both parasite and infected cells, mainly in amastigotes. Further characterization and relative quantification of the lipids altered in the infected macrophages and in the intracellular parasite *L. infantum* may contribute to clarify the pathophysiological mechanisms of visceral leishmaniasis.

Keywords VISCERAL LEISHMANIASIS; *Leishmania (L.) infantum*; lipidomics; THP-1 MACROPHAGES; LIPIDS

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O6-04: BBSOME DEPLETION IN *Leishmania mexicana* IS ESSENTIAL TO MACROPHAGE INFECTION

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The BBSome is a hetero-octameric protein complex that has an important role in cilia function and protein trafficking in eukaryotes. The complex is named after the ciliopathy Bardet-Biedl syndrome, a genetic disorder caused by mutations in one or more of the genes encoding subunits of this complex, or associated proteins. Previously, deletion of the BBSome subunit BBS1 in *Leishmania major* did not affect viability of the parasite in culture, but was required for persistence in a mouse model. In this study, the function of BBS9, a core subunit of the BBSome, was investigated within *Leishmania mexicana*. *L. mexicana* promastigotes were genetically modified to knock out the BBS9 gene (*BBS9-/-*), and to subsequently add the gene back using an overexpression vector (*BBS9-/- [BBS9]*). The *BBS9-/-* and *BBS9-/- [BBS9]* promastigotes were both less motile than WT, and had a shorter flagellum. While the viability and differentiation of *BBS9-/-* and *BBS9-/- [BBS9]* promastigotes to axenic amastigotes did not differ from WT, there was a significant decrease in the ability for *BBS9-/-* to infect THP1 cells.

Interestingly, *BBS9-/- [BBS9]* were more efficient at infecting THP1 cells. As the BBSome is implicated in protein trafficking, the surface proteome was explored using surface biotin-labelling, and the tryptic peptides were isobarically tagged using TMT11plex following pulldown with streptavidin. There was no significant difference in the surface proteome of *BBS9-/-* and WT axenic amastigotes to explain the reduction in infectivity of macrophages. Differences in the whole proteome were also investigated using stable isotope labelling with amino acids in cell culture (SILAC). The SILAC results showed a significant change in the abundance of 25 proteins between WT and *BBS9-/-* axenic amastigotes. Changes in the knockout line include a decrease in tryparedoxin peroxidase, which is implicated in defence against oxidative stress. This could explain the decrease in infectivity, as the parasites may be less equipped to survive within the harsh phagolysosome. There was a decrease in a abundance of a number of heat-shock proteins, which could indicate that the parasites are less resistant to elevated levels of cell stress. These results indicate that the intact BBSome complex is vital for *L. mexicana* infection of macrophages, and that disruption of the complex changes the proteome of the parasite significantly. The BBSome is clearly essential for the function of a number of pathways and should be considered a desirable drug target against *Leishmania spp.*

Keywords PROTEOMICS; BBSOME; OMICS; *Leishmania mexicana*; INFECTIVITY

O6-06: EVALUATION OF THE LIPID PROFILE OF INDIVIDUALS WITH VISCERAL LEISHMANIASIS COINFECTED WITH HIV

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Many patients with visceral leishmaniasis (VL), or kala-azar, and HIV/AIDS show relapsing to the recommended treatment with amphotericin B. The aim of this study was to investigate whether the reason for this phenomenon is resistance of the agent *Leishmania infantum* to the drug or whether it is due to patient factors associated with treatment. Amphotericin B susceptibility was evaluated *in vitro* using the MTT viability test with five *L. infantum* isolates obtained from HIV-infected individuals on amphotericin B. The lipid profile of 29 individuals with VL and HIV/AIDS with and without refractoriness to amphotericin B treatment was evaluated using the Analisa® (Belo Horizonte, Brazil) kit for total cholesterol, triglycerides, and HDL. The other lipid fractions (LDL and VLDL) were obtained after applying the Friedewald formula. Susceptibility to amphotericin was uniform among the five isolates tested and similar to the reference strain. Co-infected patients had normal levels of total cholesterol and triglycerides, while HDL, LDL and VLDL levels were below the reference value. VLDL, triglyceride and LDL levels were lower in relapsed VL patients than in non-relapsed VL patients. We found no evidence of amphotericin B resistance in isolates

obtained from relapsed VL and HIV/AIDS patients. However, the low serum lipid levels in relapsed VL patients opens the possibility that low lipid levels may have interfered with drug action and led to amphotericin B relapsing in VL and HIV/AIDS patients.

Keywords VISCERAL LEISHMANIASIS; HIV; AMPHOTERICIN B; DRUG RESISTANCE; LIPID METABOLISM

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O13-01: EXPLORING THE SERINOME IN *Leishmania* SPP

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Leishmaniasis are a group of diseases caused by parasitic protozoa of the genus Leishmania present in 97 reported countries with more than 12 million infected people and 350 million people at risk of infection. Treatments are available, but they are inadequate for multiple reasons, including parenteral administration, variable efficacy, toxicity, and increased resistance. New agents, with new mechanisms of action, are urgently needed to treat the disease. Given their roles in the parasite life cycle and infectivity, the serine hydrolase (SH) superfamily represents a resource to explore for new drug targets. Whilst this has been done in many systems, the Leishmania serinome remains surprisingly neglected. In this presentation we describe our work to map and explore therapeutic targets within SHs present in the Leishmania proteome using an activity-based protein profiling (ABPP) strategy. Initial experiments using commercial fluorophosphonate (FPs) probes revealed significant differences between the SH expression levels throughout their life cycles and between different *Leishmania* spp. As these probes are only effective for in vitro labelling, a suite of cell permeable probes has been synthesized and applied to study the Leishmania serinome in whole cells. Following proteome labelling and enrichment, mass spectrometry-based tagging method iTRAQ led to the identification of two serine proteases. Carboxypeptidase LmxM.18.0450 and prolyl oligopeptidase (POP) LmxM.36.6750. Using a competitive ABPP approach, we were able to identify small molecule inhibitors for these enzymes which did showed activity against both *L. mexicana* promastigotes and axenic amastigotes. Collectively, these findings suggest that the serinome is a valuable source of new drug targets and that ABPP is a reliable approach for target discovery.

Keywords LEISHMANIASIS, ABPP; SERINE HYDROLASE; PROTEOMICS; TARGET DISCOVERY

O13-03: HYBRIDIZATION AS A PHENOTYPIC ADAPTATION IN *Leishmania donovani* IS ASSOCIATED WITH CUTANEOUS LEISHMANIASIS

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Leishmaniasis is a neglected tropical disease endemic in over 90 countries. The disease has two main pathologies; cutaneous leishmaniasis (CL) that generally self-heals, and visceral leishmaniasis (VL) can be fatal if untreated. The majority of VL cases, concentrated on the Indian subcontinent (ISC) and East Africa, are caused by *Leishmania donovani*. However, recent foci of CL on the ISC have been attributed as an atypical phenotype of *L. donovani* infection including outbreaks in Sri Lanka and in Himachal Pradesh, India. Whole genome sequencing of novel isolates, and datamining of existing publicly available sequenced isolates was performed, followed by phylogenetic analysis to understand the unique phenotype of these CL causing *L. donovani* parasites. Hybridization signals were detected by heterozygous SNP analysis and where possible, determination of the parental genotypes was performed. Here we demonstrate that multiple independent hybridization events, both intra- and inter- species, involving *L. donovani* appears to have resulted in progeny with a cutaneous disease phenotype. Indeed, we identified in Sri Lanka the progeny of hybridization between *L. donovani* of Ethiopian origins and *L. major,* the same *L. donovani* and *L. tropica*. Further, we identified in India the progeny of hybridization between two ISC1 "Yeti" strains with Nepalese Highlands origins. Based on our observations first in Sri Lanka, and more recently in India, we suggest that hybridization constitutes a rapid evolutionary adaptation mechanism

to a yet unidentified environmental pressure for *L. donovani* to associate with CL.

Keywords GENOMICS; TROPISM; HYBRIDS; *Leishmania donovani;* CUTANEOUS LEISHMANIASIS

O13-04: ANEUPLOIDIES ARE AN ANCESTRAL FEATURE IN TRYPANOSOMATIDS AND COULD BE RELATED TO PARASITE ADAPTATION

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Aneuploidy, the presence of an aberrant number of chromosomes in a cell, usually results in severe abnormalities in multicellular eukaryotes as humans. However, some unicellular eukaryotes rely on aneuploidy as a mechanism to allow rapid adaptation to changing environments, having a positive fitness in stress conditions and promoting drug resistance. Aneuploidies have been largely described in protozoan parasites as *Leishmania* and *Trypanosoma cruzi*, where duplicated chromosomes vary in different hosts and can promote drug resistance. Interestingly, their closely related parasite *Trypanosoma brucei*, is mainly euploid. Hence, to evaluate if aneuploidies are an ancestral or recent feature in trypanosomatids we estimated the chromosome copy number variation in 13 Trypanosomatidae species, including *Angomonas*, *Crithidia*, *Leptomonas* and *T. vivax,* using whole genome sequencing and read depth coverage variations. Aside from the *T. brucei, T. evansi* and *T. vivax*, all the remaining species have evidence of aneuploidies, including *Paratrypanosoma confusum*, an early-branching trypanosomatid, indicating that it is an ancestral character in these parasites. The presence of aneuploidies could be detrimental in *T. brucei* clade, as their genome is packed in a lower number of larger chromosomes.

Next, we evaluated if there were consistent chromosomal duplications in the evaluated species. *Leishmania*'s chromosome 31 is constantly supernumerary, a fact reassured by our analysis of ~200 isolates from *L. donovani* and *L. infantum* populations in Africa, Asia and Brazil. This chromosome had an increased nucleotide diversity (π), which is expected, as having extra copies per cell results in more sites to be randomly mutated. Similarly, redundant copies of genes could allow a rapid adaptation and diversification without loss of function. Regarding the other trypanosomatid species, the chromosomes that have most of its genes orthologous to *Leishmania* chromosome 31 were also consistently supernumerary, even in the euploid *T. brucei* clade where regions of this chromosome are observed in two chromosomes, 4 and 8. We evaluated the function of these shared duplicated genes and we found genes involved in housekeeping functions as osmoregulation and response to stress, diverse cytoskeleton mediated processes such as cell morphogenesis, flagellar motility and cell division, energy obtaining pathways, host immune system evasion, infectivity and intracellular trafficking. We are now evaluating species-specific genes that were inserted in these duplicated regions specifically in each protozoan, as those can be important to each parasite adaptation.

Keywords ANEUPLOIDY, COMPARATIVE GENOMICS, *Leishmania*

O13-05: IMMUNO-METABOLIC PROFILING OF *Leishmania*-INFECTED MACROPHAGES (LIMS) REVEALS UNIQUE POLARIZATION AND BIOENERGETIC SIGNATURES

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Macrophages (Mφs) are the major mammalian host cells of *Leishmania* parasites. These key innate immune cells display remarkable phenotypic plasticity that permits the regulation of dynamic, cell-type-specific responses to environmental and molecular cues. They can adopt a spectrum of polarization states ranging from i) M1 Mφs that produce proinflammatory cytokines and antimicrobial oxidants, generate their energy via glycolysis and promote T helper type 1 responses to combat infections, to ii) M2 Mφs that produce anti-inflammatory cytokines, are characterized by the expression of arginase to hydrolyze arginine, generate energy via mitochondrial oxidative phosphorylation (OXPHOS), and drive T helper type 2 responses implicated in tissue homeostasis and wound healing. Several studies have shown that *Leishmania* exploits the macrophage phenotypic plasticity, driving macrophages towards an M2-like phenotype as a gateway to chronic infection. To better define this phenotype, we conducted a first, in-depth analysis of the immuno-metabolic profile of *Leishmania*-infected macrophages (LIMs). Bone marrow-derived macrophages from C57BL/6 mice were infected for 3 days with *Leishmania amazonensis* amastigotes and compared to M1 (LPS/INF□□stimulated) and

M2 (IL4/IL13 stimulated) polarized macrophages. We combined transcriptomic (RTqPCR and RNASeq) and immuno-proteomic (Proteome Profiler™ Array) analyses to respectively determine the expression of polarization markers and secretion of 111 cytokines/chemokines. Additionally, nitric oxide levels were measured using the colorimetric Griess assay, and bioenergetic profiles were monitored by real-time analyses of glycolysis and OXPHOS (ATP Rate Assay Kit, Agilent Seahorse XFe 96 analyzer). LIMs shared various expression and secretion features with M1 (increased *ccl3* expression, reduced *irf4* expression, increased VEGF secretion) and M2 Mφs (absence of TNF, IL1β, and CXCL9 secretion, and nitrite oxide production, and reduced *cd86* expression), establishing their mixed polarization profile. Aside from these polarization markers, LIMs showed unique features when compared to non-infected (M0) Mφs, including reduced expression of *rela and nfkb1*, increased expression of *igfbp3*, and secretion of GAS6, CD93, and LIX. Alternatively, LIMs showed a bioenergetics profile similar to M2 Mφs as judged by a high level of OXPHOS and an intermediate level of glycolysis (Extracellular Acidification Rate) between M0 and M1 Mφs. However, while inhibition of OXPHOS with the ATPase inhibitor oligomycin caused an important, compensatory increase of glycolysis in M2 Mφs to levels observed in M1 Mφs, the compensatory response in LIMs was strongly limited. Our data thus reveal a highly fine-tuned effect of *L. amazonensis* infection on the host cell energy metabolism that tightly controls the level of glycolytic activity, likely to limit the production of microbicidal activities. Both the steady-state energy metabolism of LIMs and the limited compensatory response was strictly dependent on live parasites as fully reversible following parasite killing with leishmanicidal Leucine-o methyl ester. In conclusion, our results uncover a unique immuno-metabolomic LIMs phenotype that seems to balance pro- and anti-inflammatory responses beneficial for short- and long-term intracellular *Leishmania* survival and growth.

Keywords *Leishmania amazonensis*; MACROPHAGES; METABOLISM; PHENOTYPE

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O13-06: PARASITE GENOTYPE STRONGLY INFLUENCES MORTALITY RISK IN VISCERAL LEISHMANIASIS

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In Brazil, *Leishmania infantum* (*syn. L. chagasi*) causes 4200-6500 cases of visceral leishmaniasis (VL) per year, 90% of the cases registered in the Americas. Screening of healthy blood donors in Brazil indicates that asymptomatic *L. infantum* infections are common, occurring in 1-6% of the general population. Life-threatening infections also occur, and the fatality rate of patients who receive treatment in Brazil is almost 10%, one of the highest in the world. The frequency of asymptomatic patients and high mortality suggests that there are parasites with varying virulence in Brazil. Since case fatality is getting worse, it is imperative to understand the factors that lead to this increased mortality in Brazil. Visceral leishmaniasis disease severity and outcomes have been associated with several host traits, as patient genotype, nutrition, age, sex, co-morbidities, and co-infections.

However, the impact of the parasite genetic variability in the disease severity is poorly understood. In this study, we examine for the first time the effects of population-wide parasite genetic variation on VL disease severity in Brazil using genome-scale methods. We quantified the effects of *L. infantum* parasite genotype on disease severity and mortality. We collected and sequenced the genomes of 109 *L. infantum* isolates from patients in northeast Brazil. We also retrieved matching patient clinical data from medical records, including mortality, sex, HIV co-infection and laboratory data (creatinine, haemoglobin, leukocyte and platelet counts). We identified genetic differences between parasite isolates, including single nucleotide polymorphisms (SNPs), small insertions/deletions (indels), and variations in genic, intergenic, and chromosome copy numbers (copy number variants, CNVs). To describe associations between the parasite genotypes and clinical outcomes, we applied quantitative genetics methods of heritability and genome-wide association studies (GWAS), treating clinical outcomes as traits that may be influenced by parasite genotype. We find that parasite genotype explains 83% chance of mortality (narrow sense heritability h^2 = 0·83±0·17), and has a significant relationship with patient sex (h^2 = 0·60±0·27). Impacts of parasite genotype on other clinical traits are lower (h^2 ≤0·34). GWAS identified 17 CNVs that were significantly associated with mortality, two with creatinine and two with bacterial co-infection, one jaundice and HIV co-infection; and two SNPs/indels that associate with age and jaundice, HIV. Hence, multiple genomic polymorphisms in the parasite appear to influence mortality and other clinical factors, consistent with variations in virulence between *L. infantum*. This is the first study to associate population-wide genetic variation in any protozoan parasite to disease severity and outcome. With a larger sample size, our GWAS approach will allow us to pinpoint the most promising gene alterations associated with VL severity, leading to a greater understanding of the molecular mechanisms of host-parasite interactions, which may be exploited for novel therapies against visceral leishmaniasis and the genetic markers discovered will be valuable to assess risks of severe disease.

Keywords *Leishmania infantum*, BRAZIL, GENOMICS

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O13-07: BIOINFORMATICS AND IMMUNOLOGICAL ANALYSIS OF THE INTERACTION OF LETIFEND® WITH DIFFERENT *LEISHMANIA* SPECIES

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Leishmaniases are a group of diseases caused by over 20 *Leishmania* parasite species. The clinical presentation and the world area affected vary as a function of the infecting parasite species. The active ingredient of LetiFend®, the canine leishmaniasis vaccine authorised in Europe, is a recombinant chimeric protein containing five fragments from four different *Leishmania (L.) infantum* proteins. Multiple *Leishmania* species are distributed in the same or adjacent geographical regions. The use of immunogens able to protect against different species has been encouraged by the WHO.The objective is a preliminary analyse, *in silico* and *in vitro,* about the efficacy of LetiFend® as a pan-*Leishmania* vaccine. Firstly, the conservation of the amino acid sequence of the five components of LetiFend® in different *Leishmania* species was studied using bioinformatics tools to predict its effect in areas where several species coexist. Also, the similarity with the human and canine genes was assessed, to evaluate the potential risk of autoimmune responses. Secondly, the quantitative and qualitative differences in the humoral immune response, after infection, were studied for different species causing canine leishmaniasis in Europe and Brazil. The *in silico* work was performed using the BLASTP tool (https://www.ncbi.nlm.nih.gov) from the National Center for Biotechnology Information, from the U.S. National Library of Medicine (NCBI). The individual sequences of LetiFend® fragments were compared with the *Leishmania* sequences in the database having a higher identity and with the human and the canine protein.
For the immunological work, the recognition of sera from six dogs infected with *L. infantum* was tested by immunoblot with soluble *Leishmania* proteins (SLA) extracted from three different *Leishmania* species. The average identity of three components of LetiFend® with the corresponding

proteins from *L. tropica*, *L. major* and *L. braziliensis* ranges between 94.2 % and 88.5 %. The average identity of the fourth LetiFend® component with the proteins from *L. tropica*, *L. major* and *L. braziliensis* is 77.6%, although the coverage of their sequences ranges between 56 and 62 % respectively. The average identity of the fifth LetiFend® component with the *L. major* protein is 100 %. The corresponding sequence is not annotated in the other species under study. The average identity of the different components with the *Canis lupus* and *Homo sapiens* proteins ranges between 34% and 49%, except for one of the fragments for which no identity was found. The immunological studies showed a similar protein profile for the four SLAs analyzed. Serum antibodies detected a very similar pattern independently of the SLA used. Furthermore, antibodies present in sera from dogs infected with *L. infantum* were blocked by *L. amazonensis* and *L. braziliensis* SLAs. The components of LetiFend® are conserved in the *Leishmania* species studied. The potential autoimmunity risk of LetiFend® is low, as its components lack a relevant identity with the human and canine homologues. The efficacy results obtained for LetiFend® could be extrapolated to *Leishmania* infection in regions were the causative agent is other *Leishmania* species like *L. amazonensis* or *L. braziliensis*.

Keywords *IN SILICO* ANALYSIS; *Leishmania* SPECIES; PROTEIN CONSERVATION; CANINE *Leishmania* VACCINE

O20-01: G PROTEIN-COUPLED RECEPTORS AS POTENTIAL INTERCELLULAR COMMUNICATION MEDIATORS IN TRYPANOSOMATIDAE

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Environmental signals sensed and transduced as stress signals, constitute a prerequisite of a successful parasite invasion; i.e., *Leishmania*, transmission, survival, pathogenesis and disease manifestation and dissemination. Diverse molecules function as inter-cellular signaling ligands. Receptors [G protein-coupled receptors (GPCRs)] and the associated transduction mechanisms are well conserved through evolution. Classical GPCR-related signal transduction systems have not yet been described in *Leishmania*, but orthologs of these molecules (with reduced domains and function) have been identified in Trypanosomatidae. These inter-cellular communication means are essential for multicellular and unicellular organism's survival. GPCRs are flexible in their molecular architecture and may interact with the so-called receptor activity-modifying proteins (RAMPs), which modulate their function, changing GPCRs pharmacology, acting as chaperones, regulating signaling and/or trafficking, in a receptor-dependent manner. Vasoactive- and neuro- peptides released into the skin in response to the noxious stimuli represented by the insect bite may trigger chemotaxis. In *L. (V.) braziliensis* the stimulated responses might be mediated by putative GPCRs (with essential conserved receptor domains). We have demonstrated the *in vitro* effect of sensory [Substance P, SP (10^{-8} M), chemoattractant] and

autonomic [Vasoactive Intestinal Peptide, VIP (10^{-10} M), and Neuropeptide Y, NPY (10^{-9} M), chemorepellent] neuropeptides at physiological levels on parasite taxis. VIP and NPY chemotactic effects are impaired by their corresponding receptor antagonists and the effect of SP is blocked by ([D-Pro 2, D-Trp7,9]-Substance P (10^{-6} M), suggesting that it might be mediated by neurokinin-1 transmembrane receptors. Additionally, we have demonstrated the chemorepellent effect of the vasoactive molecules Calcitonin Gene-Related Peptide [CGRP, (10^{-9} and 10^{-8} M)] and Adrenomedullin [AM, (10^{-9} to 10^{-5} M)], in addition to the expression of a 24 kDa band recognized by western blot analysis and (human-)-RAMP-2 antibodies. By *in-silico* research we detected a RAMP-2-aligned sequence corresponding to *Leishmania* folylpolyglutamate synthase and a RAMP-3 aligned protein, a hypothetical *Leishmania* protein with yet unknown function, thus indicating that CGRP and AM activities may be modulated by RAMP- (-2) and (-3) homologs in these parasites. Our findings suggest that proteins and molecules potentially involved in the associated receptor cascade, signpost conservation of ancient signaling systems associated with cellular responses, fundamental for cell survival, i.e., taxis and migration.

Keywords GPCRs; RAMPs; TRYPANOSOMATIDAE; CELL-CELL COMMUNICATION; SKIN NEUROPEPTIDES; STRESS RESPONSES

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O20.02: *Leishmania* ATTACHMENT IN THE SAND FLY: FROM 3D ARCHITECTURE TO MOLECULAR MECHANISMS

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Within the sand fly vector, *Leishmania* parasites have two major morphological forms, a motile promastigote with an elongated cell body and a long free flagellum, and a haptomonad, which is attached to the insect gut epithelium at the stomodeal valve through a shortened and modified flagellum. The role of the haptomonad form is not fully understood but it is likely required to maintain a persistent infection in the sand fly vector and contributes to formation of the plug, obstructing the gut and facilitating the transmission of parasites during feeding on the vertebrate host. Nevertheless, studies of the haptomonad form are limited, as this is a technically challenging life cycle form to study. Dissecting haptomonad development is critical to understand parasite transmission. To gain an in-depth understanding of the haptomonad, we first established an *in vitro* differentiation system for *L. mexicana* to generate the attached haptomonad form *in vitro*, from promastigotes. By combining two volume electron microscopy techniques – serial block face-scanning electron microscopy and serial section electron microscopy tomography – we generated high resolution 3D models of haptomonads attached to the stomodeal valve *in situ*, and compared those with models of *in vitro* differentiated haptomonads. This comparison showed that the fine ultrastructure of the attachment region is similar *in vitro* and *in situ*, which validated our *in vitro* system. Both *in vitro* and *in situ*, haptomonads were attached through the tip of a shortened flagellum, and the entire attachment interface was filled, on the flagellum side, with an electron-dense plaque of comparable size and ultrastructure *in vitro* and *in situ*. Abundant filaments and filament bundles were found inside the flagellum, reaching the attachment plaque, while the

extra-axonemal paraflagellar rod was almost entirely disassembled in the attached haptomonad flagellum, both *in vitro* and *in situ*. Using a proteomic approach to compare attached haptomonads and unattached promastigotes, we identified two proteins that are enriched at the attachment interface. Importantly, deletion of one of these proteins inhibited *Leishmania* attachment in both our *in vitro* attachment assay and in the sand fly. In this work, we dissected the structure of the haptomonad attachment interface in unprecedented detail and identified the first molecular components of this structure. Our insights into the molecular mechanisms of haptomonad attachment interface assembly are crucial to our understanding of how parasite persistence in the vector can be disrupted, potentially interrupting parasite transmission.

Keywords HAPTOMONAD; INSECT VECTOR; FLAGELLUM; DIFFERENTIATION; VOLUME ELECTRON MICROSCOPY

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O20-03: IDENTIFICATION OF ATP-BINDING PROTEINS IN *Leishmania donovani* USING A FUNCTIONAL PROTEOMICS APPROACH

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ATP-binding proteins are instrumental for many biological processes as exemplified by protein kinases that phosphorylate substrates implicated in development, cell cycle or cell survival. *Leishmania* alternates between sand fly and mammalian hosts and is able to rapidly adapt to these different environments via a differentiation process that involves ATP-binding proteins such as kinases and heat shock proteins. Here we applied ATP affinity chromatography, mass spectrometry (MS) and bio-informatics analyses on promastigote and axenic amastigote samples, with the aim to gain new insight into *Leishmania* ATP-binding proteins (ATPome) and identify potential new members that may qualify as therapeutic targets. Successive ATP affinity chromatography steps allowed us to capture all active protein kinases as judged by the absence of phosphotransferase activity in the flow though measured by *in vitro* kinase assay. MS analysis of the material eluted from the ATP beads identified 1064 proteins, including 90 kinases. We further revealed 188 hypothetical proteins that were not previously identified as ATP-binding proteins, suggesting their enrichment via interaction with ATP-binding proteins (indirect), or via binding ATP (direct) through yet uncharacterized, parasite-specific domains. We discriminated between these two possibilities using a bioinformatics approach to (i) determine *Leishmania* protein motifs specific for the ten

protein classes present in our dataset, and (ii) assess the presence of these domains in the set of hypothetical proteins, which allowed us to classify 159 of them. In particular, we identified 12 hypothetical proteins containing all 9 motifs specific of *Leishmania* kinases, suggesting that these proteins might potentially represent new members of the kinase protein family, even though their motif organisation does not correspond to protein kinases. Out of the twelve potential kinases, three could be produced as active, recombinant proteins and were confirmed as direct ATP-binding proteins by affinity chromatography. Interestingly, most of these potential novel kinases display a molecular weight above 100 kDa and thus could be moonlighting proteins with additional functions. Finally, to identify the targets of anti-leishmanial hit compounds from our past phenotypic screening, we applied our functional ATPome approach for drug target deconvolution, using the casein kinase 1 inhibitor D4476 that target casein kinase 1.2. Our results establish the first ATPome of any *Trypanosomatid* and allowed us to develop a novel pipeline combining functional proteomics and bioinformatics to annotate the *Leishmania* hypothetical proteome. Furthermore, we developed a novel target deconvolution strategy to prioritize ATP-competitive hit compounds during the screening process, and to identify their targets.

Keywords ATPOME; ATP-BINDING PROTEINS; KINASE; FUNCTIONAL PROTEOMICS; DRUG TARGET DECONVOLUTION

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O20-05: CHARACTERIZATION OF BRAZILIAN *Leishmania infantum* STRAINS REVEALS RELEVANT BIOLOGICAL AND GENETIC INTRA-STRAIN DIVERSITY

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The antiparasitic drugs available for Human Visceral Leishmaniasis (HVL) treatment in Brazil are mainly based on pentavalent antimony and amphotericin B. Some drugs, such as Miltefosine, are employed in other regions, but the success is not supported by clinical trials conducted in Brazil. However, this drug has been approved to treat Canine Visceral Leishmaniasis (CVL) in the Country, despite the fact the parasite causing HVL and CVL to be the same. *Leishmania spp*. have different intrinsic susceptibilities to drugs, grounded on factors such as the genomic plasticity of *Leishmania* and polycistronic transcription, absence of classical eukaryote gene expression regulation and chromosomal and extra-chromosomal gene copy number variation, ultimately associated with changes in protein abundance. Curiously, a recent study reported a specific genomic trait, a multi-kilobase deletion on chromosome 31 (DEL) in a wide sampling of Brazilian *L. infantum* strains, which has been associated with resistance to miltefosine and a significant reduction in ecto-3'-nucleotidase activity (an enzyme described as a relevant virulence factor). An additional feature that deserves to be explored among American *L. infantum* strains is the presence of *Leishmania* RNA virus 2 (LRV2), which might confer survival advantage to the parasite and influence drug susceptibility. We aim to perform a high-content drug screening of 200 *L. infantum* strains isolated from infected humans and dogs to identify the level of susceptibility related to drugs used in the treatment of VL in Brazil. The quantification of metacyclic promastigotes, as well as the search for LRV2, will be carried out.

The whole genome sequence (WGS) and an association of different genomic characteristics with the evaluated traits will be conducted. So far, we sequenced 195 *L. infantum* strains from 18 Brazilian states, from North, Northeast, Western, Southeast and South. As previously demonstrated by our group, DEL parasites are more frequent, representing 70,72% of the analyzed strains, and were identified in all screened regions. Conversely, in Maranhão, Mato Grosso do Sul, and Piaui Non-deleted were more frequent than DEL strains. Non-deleted parasites represented 25,13% of the strains, restrictedly detected in 6 States. Possible heterozygous strains for the deleted locus were also observed (4.10%). Differences in the growth curve were observed, revealing that the growth peak of the strains varies between 48 hs and 96 hs, suggesting that the strains start their respective logarithmic and stationary phases at different times. Nested PCR was performed to investigate the presence of LRV2, and so far, all strains tested were negative. Further *in vitro* assays to investigate infection ratios and drug susceptibility of these strains will allow us to perform correlation-tests among the obtained parameters. Together, these results highlight the inter-strain diversity among Brazilian isolates of *Leishmania infantum*. Our main goal is to contribute to elucidate intrinsic features present in *L. infantum* strains, which could represent biomarkers for susceptibility or resistance to drugs, used in the treatment of VL in Brazil.

Keywords *Leishmania infantum*; BRAZIL; VISCERAL LEISHMANIASIS

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O20-06: INOSITOL PYROPHOSPHATE (5-INSP$_7$) REGULATES NUCLEOTIDE METABOLISM IN *Leishmania mexicana*

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Inositol pyrophosphates (PP-InsPs) are second messengers that can be produced by parasitic protozoa via the action of specific InsP-kinases. PP-InsPs are involved in multiple molecular functions encompassing Pi homeostasis, vesicle trafficking, and energy dynamics. Our research is aimed at dissecting the enzymology of the pathway toward the formation of PP-InsPs as well as defining the functional role of the most abundant stereoisomers found in *Leishmania* parasites. Using an LC-MS/MS method tailored for the analysis of InsPs we have identified 5-InsP$_7$ as the most abundant PP-InsP in *Leishmania* cells. The 5-InsP$_7$-producing enzyme (inositol hexakisphosphate kinase: IP6K) has been shown to be functional in these parasites and a conditional KO (using Dicre system) for this gene showed slower proliferation rates in promastigote forms. This growth phenotype has been rescued by adding a photocaged 5-InsP$_7$ reagent, suggesting a direct role for 5-InsP$_7$ in parasite survival. Rapamycin-induced IP6K ablated parasites displayed higher adenylate energy charges (AEC) as compared to uninduced (dmso) cells. To further analyse the influence of 5-InsP$_7$ on energy expenditure of *Leishmania*, we are investigating the function of a unique SPX domain-containing ribophosphokinase identified in kinetoplastids. SPX domains are a protein interface that mediates specific binding to PP-InsPs working as allosteric regulators. Parasites devoid of the

gene Lmx*PRPS4* displayed lower AEC and the inclusion of a mutation at the SPX domain resulted in lower proliferation rates and impaired virulence in mice model. Our data suggest 5-InsP_7 influences energy dynamics in *Leishmania* and its interaction with an enzyme of nucleotide biosynthetic metabolism (via SPX) establishes a direct link for this function. This metabolic intersection can be informative to better understand our knowledge of the appearance of persistent forms of *Leishmania.*

Keywords INOSITOL PYROPHOSPHATES, RIBONUCLEOSIDE 5'-PHOSPHATES, SPX DOMAIN, IP6 KINASE

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O26-01:
QUIESCENCE IN *Leishmania* PROMASTIGOTES AND AMASTIGOTES AS AN ADAPTIVE STRATEGY TO STRESS: REMODELING OF TRANSCRIPTOME AND METABOLOME

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Microorganisms can adopt a quiescent physiological condition which acts as a survival strategy under unfavorable conditions. Quiescent cells are characterized by reversible slow or non-proliferation and a deep downregulation of processes related to biosynthesis. Although quiescence has been described mostly in bacteria, this survival skill is widespread, including in eukaryotic microorganisms such as *Leishmania*. It is believed to play a role in asymptomatic infections, and therapeutic failure without the associated development of drug resistance. Despite of this, very scarce studies have pursued the understanding of this resilient state. Several knowledge gaps remain, including: i) experimental evidence of quiescence as parasite's strategy to survive drug pressure, ii) understanding of the mechanisms of quiescence's maintenance, iii) the degree to which quiescent cells derived through different environmental insults may share molecular and metabolic traits, and iv) the similarity between quiescent traits shown in both *Leishmania* promastigote and amastigote stages. In our *in vitro* study,

we used axenic promastigotes and amastigotes of a *Leishmania lainsoni* line highly susceptible to PAT (IC50= 0.1 µg/mL) and studied them under 2 conditions of stress, stationary phase (STA) and exposure to potassium antimonyl tartrate (PAT). Subsequently, we quantified the transcriptome and metabolome of quiescent promastigotes and amastigotes induced under PAT and STA conditions. We found that in PAT (1 µg/mL ~10x IC50) and STA conditions, both *Leishmania* stages enter a quiescent state characterized by negligible proliferation, low expression of the ribosomal RNA locus, and decreased mitochondrial activity. Remarkably quiescent cells were able to endure up to 90 fold the PAT IC50 (9 µg/ mL). Upon removal of the drug pressure, the survivors resumed their proliferation to generate a new population that remained highly susceptible to the drug (IC50= 0.05 µg/mL). At the transcriptomic level, quiescent cells had a very diminished transcriptome size, with levels dropping to as low as 0.4% of those in proliferating cells and impacting the levels of the vast majority of mRNAs. Gene set enrichment analysis indicated several processes related to biosynthesis were downregulated, among them: oxidative phosphorylation, the synthesis of amino acids and aminoacyl-tRNAs, the fatty acids metabolism, and the TCA cycle. All quiescent populations shared a few upregulated transcripts encoding membrane components, such as amastins and GP63, or processes like autophagy. The metabolome followed a similar trend of overall downregulation, albeit to a lesser magnitude than the transcriptome. Noteworthy, among the commonly upregulated metabolites, we found those involved in carbon sources as an alternative to glucose. This study shows quiescence as a mechanism deployed by *Leishmania* to overcome harmful conditions as those found under antimonial drug pressure and shows commonly modulated features at transcriptomic and metabolic levels across stimuli and stages.

Keywords QUIESCENCE; DRUG PRESSURE; STATIONARY PHASE, SURVIVAL; TRANSCRIPTOMICS; METABOLOMICS

O26-02: *Leishmania donovani* INFECTION LEADS TO TRANSLATIONAL REPROGRAMMING OF MTOR- AND EIF4A-SENSITIVE IMMUNE-RELATED TRANSCRIPTS IN MACROPHAGES

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Protozoan parasites of the *Leishmania donovani* complex are the causative agents of visceral leishmaniasis, a chronic infection that is fatal when left untreated. The lack of efficient vaccines and the failure to control emerging parasite resistance reflect the urgent need for a better understanding of the molecular mechanisms that regulate the interactions between *Leishmania* spp. and the host. Regulation of mRNA translation efficiency can constitute a host defense mechanism during infections but can also be a process exploited by the invading pathogen. It is well-documented that *L. donovani* affects transcription to subvert host cell functions. However, discrepancies between transcriptomic and proteomic data of *L. donovani*-infected cells suggest that post-transcriptional regulatory mechanisms also contribute to modulate host gene expression programs. Using polysome-profiling quantified by RNA-Seq, we found that one third of protein-coding mRNAs expressed in macrophages are differentially translated upon infection with *L. donovani* (1,520 and 1,607 mRNAs up- or down-regulated, respectively). Gene ontology analysis identified key biological processes enriched for translationally regulated mRNAs and were predicted to be either activated (*e.g.*, chromatin remodeling and RNA metabolism) or inhibited (*e.g.*, intracellular trafficking and antigen presentation) upon infection. Mechanistic *in silico* and biochemical analyses showed selective activation mTOR- and eIF4A-dependent mRNA translation, including transcripts encoding central regulators of mRNA turnover and inflammation (*e.g.*,

PABPC1, PKR, TGF-β, etc.). Intriguingly, *L. donovani* survival within macrophages was favored under mTOR inhibition but was dampened by pharmacological blockade of eIF4A. Overall, this study uncovers a vast yet selective reprogramming of the host cell translational landscape early during *L. donovani* infection and suggests that some of these changes are involved in host defense mechanisms while others are part of parasite-driven survival strategies. Ongoing research in our laboratory using *in vitro* and *in vivo* models of *L. donovani* infection will shed light on the contribution of mTOR- and eIF4A-dependent translational programs to the outcome of visceral leishmaniasis.

Keywords *Leishmania donovani*, MACROPHAGE; mRNA TRANSLATION; mTOR; eIF4A

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O26-03: TRANSCRIPTOME PROFILE OF *Leishmania major* IN RESPONSE TO HEME LIMITATION REVEALS NEW ESSENTIAL GENES REQUIRED FOR PARASITE VIRULENCE

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Heme is an iron-coordinated porphyrin essential in most aerobic organisms. Trypanosomatid parasites such as *Leishmania* are auxotrophic for heme since they lost its complete biosynthesis pathway during evolution and must obtain this essential compound from the infected host. Exploiting this heme dependency is a rational way to find new leishmanicidal agents. The aim of this work is to identify and characterize genes/proteins differentially regulated by heme in *Leishmania major*, and evaluate their potential use as new drug target. First, we analyzed the transcriptomic differences between *L. major* promastigotes cultured in the presence or the absence of heme by RNA-seq. Samples were sequenced in an Illumina Nextseq 550 using a standard protocol for paired-end 75nt libraries and sequence data generated as fastq files were analyzed using miARma-Seq. Reads were aligned against *L. major* Friedlin reference genome from TriTrypDB version 31 and summarized into gene expression levels using Feature Counts. Differentially expressed genes (DEG) were detected by edgeR with a false discovery rate-adjusted *p* value (FDR) <0.05. Our preliminary results show 1,908 DEG (802 up-regulated in the absence of heme and 1,106 down-regulated). 12 DEG were selected for qPCR validation (6 up-regulated and 6 down-regulated). In order to perform a functional interpretation of the results, gene ontology (GO) terms of each of these genes were extracted from

TriTrypDB database and a functional enrichment analysis was carried out. Among the most enriched terms (having a p-value <0.05) in the GO processes we found “regulation of developmental process” and “transmembrane transport” and among the molecular functions ontology “heme binding” and “metal ion transmembrane transporter activity” were highly represented. Some of the most up-regulated genes in the heme depletion condition were selected for further analysis. We first evaluated the subcellular localization of selected genes by CRISPR/Cas9-mediated C-terminal *in situ* tagging of the ORF with a fluorescent tag. Heme-regulated proteins were located in different subcellular compartments, from plasma membrane to the mitochondrion. In addition, we generated knockout parasites (deleting one or both alleles) of each selected gene using the CRISPR/Cas9 technology and evaluated their essentiality, their ability to differentiate into infective metacyclic parasites, their ability to infect and replicate within macrophages cultured *in vitro*, and also their virulence *in vivo* in a cutaneous leishmaniasis mice model. Some of the genes were essential in the promastigote stage while others were not, allowing homozygous KOs to be obtained. We also identified essential genes in the amastigote stage, since the deletion of one or both alleles of the gene prevented their development as intracellular amastigotes in *in vitro* infection assays, as well as the development of the disease in animal models. Our results suggest that several heme-regulated proteins are crucial for parasites survival in the mammalian host and therefore could be candidates to become new therapeutic targets.

Keywords: *Leishmania*; HEME; DRUG TARGET; OMICS

O26-04: A NOVEL AXENIC DIFFERENTIATION MODEL BRINGS NEW INSIGHTS INTO QUIESCENCE IN *Leishmania*

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It is speculated that quiescence of amastigotes underlies *Leishmania* persistence and explain treatment failure in absence of drug resistance, relapse several years after the original infection or the high prevalence of asymptomatic cases. Through different experimental approaches applied to different *Leishmania* species it was demonstrated that quiescent amastigotes are characterized by both negligible rate of proliferation, reduced bio-energetic level, a strongly altered metabolism, and strongly decreased level of rRNA expression with a drastic transcriptional shift. The mechanisms of quiescence likely result from an adaptative process during the evolution of Trypanosomatids, to face harsh (micro-)environmental conditions. Information regarding the environmental triggers and the parasite-derived factors that determine *Leishmania* amastigotes entry into quiescence, maintenance or exit from this state is very limited. A main limitation to study natural quiescence is the scarce number of parasite persisters cells in a large number of host cells, where these amastigotes would have exiguous metabolism and very low gene expression. Moreover, there are evidences that quiescent amastigotes co-exist with non- quiescent ones, leading to an heterogeneous population within/among host cells. Here we present a novel axenic model for triggering *Leishmania* quiescence that was highly reproducible across independent experiments and that will

support the investigation of the cellular and molecular mechanisms underlying quiescence in *Leishmania*. We used a negative biomarker that turned off during quiescence (GFP inserted in ribosomal RNA locus) to demonstrate that nutrient starvation and hypoxia induces over 95% and up to 100% of *L. mexicana* quiescent amastigotes. This is a considerable improvement if compared with the traditional axenization models by pH and temperature shifts that produces amastigote populations containing 30 to 45 % of quiescent cells. We undertook a targeted gene expression study that compares quiescent amastigotes with non-replicative stationary promastigotes. We found that amastins AMA 8A and AMA 34D were significantly overexpressed in amastigotes under hypoxic conditions with respect stationary promastigotes. In contrast DRDB3 gene encoding a protein related to mRNA stability was significantly downregulated under hypoxia. These genes are therefore protein biomarker candidates of the amastigote quiescent stage using our developed nutrient starvation and hypoxic stress axenic cultures. On the other hand, PNC and LIT1-4 gene encoding nicotinamidase and iron transporter proteins respectively, were very stable in all stage culture conditions suggesting their metabolic relevance throughout parasite differentiation, an observation that they might be considered as potential drug targets. Results were completed by an untargeted transcriptomic approach (bulk RNAseq): sequencing data are currently under processing and will be presented at the conference.

Keyword *Leishmania mexicana*; QUIESCENCE; AMASTIGOTE; TRANSCRIPTOMIC; RNA-SEQ

Financing Belgian Directorate General for Development Cooperation-DGDC (framework agreement 4)

O26-06: LEISHMANIA SPECIES IN THAILAND: ANALYSIS OF *Leishmania orientalis* ISOLATE PCM2 (FORMERLY NAMED *Leishmania siamensis*) AND *Leishmania martiniquensis* ISOLATE PCM3 FROM THAILAND'S SOUTHERN PROVINCE

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Leishmaniasis is caused by an intracellular kinetoplastid protozoan *Leishmania* in tropical and subtropical areas. Before 1999, leishmaniasis cases in Thailand were considered imported causes. To date, *Leishmania martiniquensis* and *Leishmania orientalis* are recently found as species, which have caused autochthonous leishmaniasis in Thai patients as emerging autochthonous leishmaniasis in Thailand. In this study, *L. orientalis* isolate PCM2 and *L. martiniquensis* isolate PCM3 from Thailand's southern province were analyzed using next-generation sequencing data compared with the other 14 *Leishmania* species. Draft genome of *L. orientalis* Isolate PCM2 (30.01 Mbp) and *L. martiniquensis* PCM3 (32.39 Mbp) were constructed by *de novo* and reference-based assembly method, respectively. To investigate variation between isolates, *L. orientalis* isolate LSCM4 and *L. martiniquensis* isolate LSCM1 from northern provinces were used to analyze. The comparison to the northern isolates' genomes demonstrated similarities between these isolates with a level of genome and

proteome diversity, implying the presence of separate strains. Comparative proteome analysis revealed six distinct protein groups with 53 distinct proteins in strain PCM2 and 97 distinct proteins in strain PCM3. Several proteins were shown to be associated with virulence, resistance to drugs, and stress response. Thus, the findings may imply the need for additional genetic and population genomic research, as well as close monitoring of *L. orientalis* and *L. martiniquensis* in Thailand and neighbouring locations.

Keywords *Leishmania orientalis*; *Leishmania siamensis*; *Leishmania martiniquensis*; COMPARATIVE GENOMICS; BIOINFORMATICS; LEISHMANIASIS

O27-02: CRISPR/CAS9 TRANSPORTOME GENETIC SCREEN IN *Leishmania mexicana* IDENTIFIES MEMBRANE TRANSPORTERS IMPORTANT FOR PARASITE SURVIVAL

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Molecular transport is integral to all biological processes; thus, transporter proteins play fundamental roles in cell biology, facilitating the translocation of ions, nutrients, metabolites and waste products. Their gatekeeper function is crucial for cellular physiology under changing conditions as well as relevant for drug delivery and resistance development. We combined examination of the L. mexicana genome annotation, basic bioinformatics and literature searches to identify a predicted 'transportome' of 315 proteins. The entire predicted transportome was targeted with CRISPR/Cas9 (LeishGEdit.net), to generate a library of barcoded gene deletion mutants. Of the 315 genes targeted, 190 yielded viable promastigote null mutants and 33 further incomplete gene deletion mutants

were recovered (including heterozygous KOs), whilst 92 appeared to be refractory to deletion. Viable mutants were pooled and their growth/survival was assessed under four different conditions: (i) standard in vitro culture in M199 medium, (ii) exposure to miltefosine in vitro, (iii) infection of human induced pluripotent stem cell derived macrophages co-cultured in vitro and (iv) mice infection, using an in vivo footpad model of infection. Illumina sequencing of PCR amplicons amplified from genomic DNA was used to determine changes in barcode abundance and quantitation of mutant fitness. The pools of mutants displayed a broad spectrum of growth/survival phenotypes associated with the distinct conditions examined. Interestingly, seven mutants (deletions of MC, VIC, AAAP, CaCA2, MC and ABC family transporters) showed significantly reduced growth rates and some motility defects in promastigotes. A distinct cohort of mutants, including a mitochondrial iron transporter, a calcium-activated potassium channel and a Golgi Mn2+-Ca2+/H+ exchanger showed loss of fitness in the mouse infection model. Loss of V-ATPase components consistently showed severe defects both in mouse infections and in in vitro macrophage infections. In a positive selection assay, exposure to miltefosine was used to identify mutants with higher drug tolerance. This confirmed that deletion of the miltefosine transporter (MT) and its subunit Ros3 rendered cells resistant and showed that none of the other transporter deletions in the library conferred comparable levels of resistance. This work helps to elucidate the contributions of transporter related proteins to Leishmania biology, by evaluation of the relative fitness of each mutant in vitro and in vivo, in bar-seq fitness screens. Furthermore, this transportome knockout library provides a novel resource to study the function of Leishmania transporters in different environments, identify transporters essential for survival and their contributions to mechanisms of drug resistance.

Keywords CRISPR/Cas9; BAR-SEQ; TRANSPORTERS; *Leishmania mexicana*; DRUG RESISTANCE

Financing The UK Medical Research Council, a Marie Curie Individual Fellowship to AAW, the Royal Society and the Wellcome Trust

O27-03: ANALYSIS OF CHROMATIN DENSITY AND RNA SYNTHESIS USING ATAC-SEQ AND PRO-SEQ IN THE CONTEXT OF STAGE DIFFERENTIATION IN *Leishmania donovani*

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Leishmania spp. undergo two-phased life cycle: elongated, flagellated promastigotes in the insect gut, and non-flagellated, ovoid amastigotes in mammalian host cells. Stage conversion is central to establishing infection. Differentiation is triggered by elevated temperature and acidic milieu, but can also be triggered by inhibiting HSP90 with radicicol. Ribosome profiling analyses in *L. donovani* revealed the changes in protein synthesis between promastigotes and amastigote-like forms induced by HSP90 inhibition and showed increased histone synthesis. Using micrococcal nuclease analysis, we confirmed that increased histone synthesis is associated with denser chromatin in axenically induced amastigotes, as well as in radicicol-induced amastigote-like forms. We also mapped genome-wide chromatin density in *L. donovani* using an Assay for Transposase Accessible Chromatin and sequencing (ATAC-seq), the first application of this method in *Leishmania* spp.. ATAC-seq uses a Tn5 transposase to fragment the DNA in open chromatin regions, simultaneously tagging fragments for Illumina sequencing, resulting in reads from open chromatin regions only. The data showed that regions of transcription initiation, both in divergent strand switch regions (dSSR) and in 5'-telomere regions where transcription of polycistronic transcription units (PTUs) initiates, are associated with denser chromatin in axenic amastigotes and in HSP90-induced amastigote-like forms but not in highly proliferative promastigotes. Subsequently, we analysed the impact of chromatin density on transcription in *L. donovani*. We established a precision nuclear run-on and sequencing (PRO-seq) for the analysis of RNA synthesis rates. PRO-seq was first used in *Drosophila*

melanogaster and is based on a nuclear run-on reaction with biotinylated NTPs, the incorporation of which leads to the termination of transcription, allowing the position of RNA polymerase complexes and nascent 3' RNA ends to be mapped. To our knowledge, the use of PRO-seq in this project is the first application in Trypanosomatida and gave us detailed information about the distribution of RNA polymerase complexes over the *L. donovani* genome, analysing life cycle stage-specific changes. We were able to confirm polycistronic, constitutive transcription of PTUs within and between coding regions. Furthermore, we analysed all divergent SSRs and convergent SSRs of promastigotes, axenic amastigotes and radicicol-induced amastigote-like forms in high detail to gain insight into transcription initiation and termination. We found that transcription initiates sites are not affected by stage conversion, suggesting the presence of preferred transcription initiation points. In addition, we found evidence of paused RNA polymerase complexes, which are now under further analysis. Lastly, the observed changes of chromatin density have no obvious impact on the initiation and elongation by RNA polymerases.

Keywords TRANSCRIPTION INITIATION; POLYCISTRONIC TRANSCRIPTION; HSP90; CHROMATIN; NUCLEAR RUN-ON

O27-04: HIGH THROUGHPUT SINGLE-CELL GENOME SEQUENCING GIVES INSIGHTS INTO THE GENERATION AND EVOLUTION OF MOSAIC ANEUPLOIDY IN *Leishmania donovani*

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Aneuploidy, i.e., an imbalance in the copy number of chromosomes in a cell, is a ubiquitous feature of *Leishmania.* In this organism, chromosome copy number (CCN) alterations represent an adaptive mechanism, modulating gene expression and possibly impacting phenotypes. Moreover, variations in CCN within single parasites in clonal populations was previously observed in a small subset of chromosomes using fluorescence hybridization methods. This phenomenon, termed mosaic aneuploidy (MA), have important evolutionary and functional implications which remains under-explored. We initially applied and validated a high throughput single-cell genome sequencing method to study for the first time the extent and dynamics of whole karyotype heterogeneity in two *Leishmania donovani* clonal populations representing different stages of MA evolution in vitro. In these two populations, we identified 117 and 208 different karyotypes co-existing among 2378 and 1516 promastigotes respectively. We observed that drastic changes in karyotypes quickly emerge in a population stemming from an almost euploid founder cell. The presence of polyploid cells at early stages suggests that these initial drastic changes may be generated by polyploidization/hybridization followed by assorted ploidy reduction, as

has been observed in yeasts. During further stages of expansion, MA increases by moderate and gradual karyotypic alterations. We also observed that MA usually affected a defined subset of chromosomes, of which some display an enrichment in snoRNA genes which could represent an adaptative benefit to the amplification of these chromosomes. To gain insights into the first molecular events during MA emergence and identify potential drivers, we set up new series of experiments in which we follow the populations derived from euploid (except for chromosome 31) and aneuploid subclones. These subclones are analyzed by (single cell) genome sequencing during early expansion and (ii) by single cell RNAseq at passages 1-5, a time span where transitions from euploidy to aneuploidy were observed in bulk. Data analysis is currently running and will allow a unique integrated insight to be presented at the conference. Our data provide the most complete characterization of MA in *Leishmania* and pave the way for further functional studies.

Keywords ANEUPLOIDY; MOSAICISM; SINGLE-CELL GENOMICS; KARYOTYPE HETEROGENEITY

O27-05: A FOLLOW-UP OF A NEGLECTED TROPICAL DISEASE: OMICS IN LEISHMANIASIS AT INMEGEN, A NATIONAL INSTITUTE OF HEALTH IN MEXICO

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In Mexico, Leishmaniasis has been detected in at least 13 states. The most predominant clinical form of the disease is localized cutaneous leishmaniasis (LCL), however, there are cases of visceral (VL) and diffuse cutaneous leishmaniasis (DCL) also reported. *Leishmania mexicana - the parasite* - causes LCL and DCL nevertheless infections with *L. tropica* as an imported cases and *L. braziliensis* has been reported in Chiapas state, in the southeastern of Mexico. For the past 13 years the National Institute of Genomic Medicine (INMEGEN) in Mexico under the "*Leishmania* project" has

been studying the relationships between host, vector and parasite. Using different OMICs we have questioned host and parasite. Gene expression on NK human cells, whole exome sequencing, time course gene expression and microRNA profiling was carried out on host (assays *in vitro* to follow the infection during the first 48 hours in human macrophages). Proteomics, whole genome and transcriptomics were used on isolated parasites from the lesions. Our results showed that IL-1 SNP (-511 C/T) variant may contribute to the risk of developing the disease in patients infected with *L. mexicana*. Additionally, an increased *in vitro* production of IL-1 by monocytes and an increased serum expression of the cytokine correlates with the disease severity since it was significantly higher in DCL patients heavily infected. Differences in the NK-cell response towards *L. mexicana* lipophosphoglycan between patients with LCL and DCL were identified through gene expression profiling. Our group reported that studies conducted in time series could be far more informative than those that only capture a specific moment in time. Taking this idea, experiments using human macrophages infected with parasites with low or high index of infection during 3, 6, 18, 24 and 48 hours to analyze the expression of mRNAs and miRNAs were performed. Whole genome sequencing, whole transcriptome by RNA-Seq as well as proteome of *L. mexicana* parasites isolated from lesions of LCL and DCL patients have also been conducted. Results showed differences in gene expression and protein profiles between both groups. In addition to the genome assembly of *L. mexicana*, we were able to obtain the sequence of 10 genes of the parasite's maxicircle and with this, we design new molecular targets for the diagnosis of leishmaniasis. These studies in Mexico aim at understanding the molecular basis of infection and the disease. However, epidemiological triad of leishmaniasis are scarce, and for this reason it has been considered a neglected tropical disease (NTD) there is no impulse in research that allows generating new hypotheses to fully understand the leishmaniasis. An important goal for us is to collaborate with different groups in our country such as Yucatan, as well as other countries. Thus far, we have strengthened our collaborative work with Brazil and Italy. The effort made at INMEGEN, a National Institute of Health, studying a NTD represents a substantial advance for our group proposing a roadmap for the future to help patients with this and other NTDs to be accurately diagnosed and treated with low level toxicity drugs.

Keywords MICROARRAYS; SNPs; GENOMICS; TRANSCRPTOMICS; PROTEOMICS

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O27-06: VEUPATHDB.ORG: POPULATION BIOLOGY, OMICS DATA AND BIOINFORMATIC TOOLS FOR *Leishmania*, OTHER KINETOPLASTIDA AND THEIR VECTORS

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VectorBase and TriTrypDB are part of the VEuPathDB project, a bioinformatics resource center (BRC) focused on eukaryotic and fungal pathogens, vectors, and host informatics. VectorBase and TriTrypDB have 58 and 81 genomes respectively, including *Lutzomyia longiplapis; Phlebotomus papatasi*; 30 *Leishmania* and 42 *Trypanosoma* strains; and species from other kinetoplastids; new genomes are made available in bi-monthly releases. Other omics data include transcriptomes, proteomes, and genetic variation (SNPs). In addition, our internal pipelines analyze a wide variety of omics data and couple the analysis results to data mining capabilities, data visualizations, and custom tools to facilitate the discovery of meaningful relationships from large volumes of data. Sequencing data can be queried against *in silico* predictions such as domains, homology, and pathways. VEuPathDB empowers users to leverage omics data without the need for computational programming. Data mining strategies include records that compile all data for one or a few genes or a whole genome. Users might begin at a gene page to view tables and graphs of that gene's behavior in an RNA-Seq experiment, then transition to the genome browser for a dynamic view of the RNA-Seq data aligned to the genome, as well as the opportunity to view nearby gene models or other data types. The search strategy system allows users to query from a genome-wide perspective, easily merge evidence from diverse data and across species, and ask questions such as 'Find genes in *Leishmania* species X that are expressed in promastigotes and amastigotes, that cause cutaneous and visceral leishmaniasis' or 'Find me all *Leishmania* isolates from North America' or

'Find me *Leishmania* X genes that have an epitope and evidence of expression at the protein level '. Users with their own omics data can use Galaxy to privately analyze and port their results to VEuPathDB for comparison. Population biology data can be queried in an interactive map tool (MapVEu), from which raw data sets can be downloaded. The MapVEu tool includes population abundance, pathogen infection status, insecticide resistance (genotypes and phenotypes), and blood meal host data among others. MapVEu currently includes sandflies and other arthropods. Our active user support offers an email help desk, FAQs, social media, tutorials, webinars, and workshops. Email us at help@veupathdb.org with questions or suggestions.

Keywords OMICS; BIOINFORMATICS; MOLECULAR BIOLOGY; POPULATION DATA; OPEN ACCESS

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O33-01: BENCHMARKING PRESERVATION METHODS FOR *Leishmania* SP. SINGLE-CELL RNA-SEQ STUDIES

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Recent advances in single-cell technologies have dramatically increased the resolution at which gene expression can be studied. Given the high infrastructural requirements for running those technologies, direct analysis of clinical or environmental samples is mostly not feasible, implying the need for preservation methods with minimal impact on the integrity of the transcripts. Where benchmark analyses on preservation of samples for single-cell transcriptomics already exist for organisms like human and mice, such analysis is missing for protozoan species like *Leishmania.* We investigated the potential impact on the quality of *Leishmania* single-cell RNA-seq data for three different preservation methods, i.e. cryopreservation using either glycerol or dimethyl sulfoxide (DMSO) as cryoprotectants, and methanol fixation. Fresh cells were used as golden standard. In vitro *Leishmania donovani* promastigotes were prepared as a mixture of 4 different time points representing different stages in culture (day 1, 3, 5 and 7), mixed in equal amounts, and split in one aliquot for each method. Each aliquot was processed with the 3' Single-cell Gene Expression solution (10x Genomics) and subsequently sequenced on the Illumina NovaSeq platform. Sequencing data were mapped to the reference genome using the single-cell implementation of STAR, and downstream data processing was performed in R using the Seurat package. General Quality Control parameters like number of cells recovered, amount of reads per cell or number of genes detected per cell did not show aberrant results in preserved samples. Using a pseudobulk approach, summing all reads per gene over all cells, the correlation of the gene expression values between fresh cells versus DMSO and glycerol cryopreserved samples was high, i.e.

0.97 and 0.96 respectively. However, the correlation value calculated between fresh and methanol preserved cells dropped to 0.76. Interestingly, the correlation between DMSO and glycerol preserved samples was 0.99, suggesting that the transcriptome is affected in the same way by both methods. As preservation might affect cells in distinctive life stages in a different way, a clustering of the single-cell transcriptomes was performed to group cells potentially in the same life stage, and the correlation analysis mentioned above was repeated on a per-cluster basis, confirming the better correlation between DMSO or glycerol versus the fresh samples. Lastly, checking the differentially expressed genes between the preserved samples and the fresh cells – which should be as close to 0 as possible – confirmed our earlier observations: the cryopreserved samples only had a limited number of differentially expressed genes (12 and 22 for DMSO and glycerol respectively, of which 5 shared between both methods), versus 147 for methanol preserved cells. Our preliminary results show that DMSO or glycerol cryopreservation has only a minor impact on the quality of the scRNA-seq data produced, while methanol fixation should be avoided. Further analysis of the single-cell transcriptome landscape should elucidate whether differences in gene expression between preservation methods correspond to particular biological processes, like stress or heat shock response. Potentially, biomarkers can be identified which can be used in future samples as quality control parameter to check the quality of the preservation process.

Keywords SINGLE-CELL TRANSCRIPTOMICS; PRESERVATION; SEQUENCING; BIOINFORMATICS

O33-02: IMMUNOGENETIC BASIS OF DOGS EXPOSED TO BIOLOGICALLY DISTINCT GENOTYPES OF *Leishmania infantum*

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Investigating the host's genetics underlying the outcome of visceral leishmaniasis (VL) has identified substantial amounts of biosignatures, which has contributed to a better understanding of the VL pathogenesis. However, the impact of the interplay of the parasite and host genotypic variation during the natural infection over the VL epidemiology has been little investigated. Previous results grounded the hypothesis that *Leishmania infantum* strains presenting a 12k deletion in chromosome 31 and reduced virulence *in vitro* lead to a mild infection with lower parasite load in the main reservoir, the canine host. Such animals would remain for longer periods under-detected by the diagnostic methods and, thus, can be a source of infection, contributing to the spread of deleted *L. infantum* genotype (DEL). To test this hypothesis, we investigated clinical,

parasitological, and immunogenetic parameters of domestic dogs exposed to distinct genotypes of the parasite. We analyzed 48 dogs living in urban areas of Bauru, Caieiras, Embu, Fernandópolis, Itapira, Jales, and Votuporanga Municipalities in the Western of the São Paulo State, Brazil. The diagnoses were screened by Dual-Path Platform (DPP) and confirmed by enzyme-linked immunosorbance assay (ELISA) and polymerase chain reaction (PCR). Quantitative PCR (qPCR)-based genotyping of lymph node biopsies indicated that 29/48 animals were infected by DEL and 19/48 non-deleted strains (Non-DEL). No association was observed between parasite genotypes and the clinical forms (subclinical infection/diseased; $p = 0.3801$) or clinical progression of canine VL (CVL), ($p = 0.0764$). Nevertheless, the lymph node parasite load (qPCR, $p = 0.0035$) and the anti-*L. infantum* IgG levels (ELISA, $p = 0.0374$) were lower in dogs infected by DEL vs. Non-DEL strains. These represent more robust parameters to assess the progression of the natural *L. infantum* infection in dogs. Thus, these results support our main hypothesis. We further investigate the possible association between the immunogenetics underlying the *L. infantum* infection in dogs and the *L. infantum* DEL/NonDEL genotypes. A total of 173,685 SNP markers were genotyped in whole blood samples by Illumina CanineHD beadchip. The animals' genotypes were determined based on SNP markers previously described as protective and non-protective based on association with i) lymph node parasite load, ii) clinical progression, iii) anti-*L. infantum* IgA, IgM and IgG, and iv) anti-*Lutzomyia longipalpis* salivary gland homogenate (SGH) IgG responses. The chi-square test showed association between dogs infected with DEL and the allele A of the SNP BICF2G630654815 ($p < 0.0001$) – associated with low IgG levels, and allele B of the SNP BICF2P397161 ($p < 0.0001$) – associated with high levels of IgM. In our previous reports, both alleles were described as markers for a protective profile. Taken together, our findings corroborate the hypothesis that strains of *L. infantum* carrying a deletion in chromosome 31 establish a milder and less detectable infection, potentially contributing to the maintenance of the VL transmission.

Keywords CANINE VISCERAL LEISHMANIASIS; *Leishmania infantum* GENOTYPES; CANINE IMMUNOGENETICS, SNP MARKERS

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O33-03: ELUCIDATING HOST AND PARASITE FACTORS INVOLVED IN QUIESCENCE OF *Leishmania mexicana*

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Drug treatment options for Leishmaniasis in the clinical setting remain limited, and treatment failure is reported widely. Both host and parasite factors play a role in treatment outcome; understanding this interplay will be important for predicting treatment outcome. One current hypothesis is that persistent *Leishmania* survive immune and drug challenges and cause re-emergence of active disease. These persistent parasites may demonstrate a quiescent phenotype, defined by a state of temporary growth arrest and repressed metabolism. To investigate the role of quiescent parasites in treatment failure and identify markers of quiescence, we study two model systems of quiescence in *Leishmania mexicana.* First, we have established a long-term macrophage infection model in murine bone marrow-derived macrophages in which quiescent *L. mexicana* amastigotes can be observed up to 2 weeks after infection using Cell tracer dye and Bromodeoxyuridine labelling, through automated fluorescence microscopy analysis. In order to elucidate the host factors involved in *Leishmania* quiescence we have manipulated the activation state with IL-4 and INFγ of infected macrophages to observe changes in the emergence of *L. mexicana* quiescence. Through this we aim to identify immunological determinants involved in parasite quiescence. Second, we use purine-starvation to induce a quiescent-like state in promastigotes. After 2 days of purine-starvation *L. mexicana* undergo a temporary state of growth arrest that can be maintained for weeks and reversed by providing a purine source. Using this model, we have

performed metabolomic, single-cell transcriptomic and proteomic analysis to elucidate pathways associated with this induced quiescence, exploiting CRISPR-Cas9 technology to disrupt these pathways and elucidate their role as putative markers of the quiescent state. Moreover, both systems are used to investigate if quiescent *L. mexicana* resist treatment with anti-leishmanial compounds used in the field. Our work showed that macrophage activation status does not have an impact on the emergence of quiescent parasites suggesting that induction of quiescence *in vitro* is a stochastic rather than an induced process. Differences in susceptibility between quiescent and proliferating *Leishmania* to anti-leishmanial drugs was observed. We thereby indicate a role of quiescent parasites in drug treatment failure. Multi-omic analysis of quiescent promastigotes highlighted dramatic differences in metabolism and the proteome of quiescent parasites that may offer markers of quiescence as well as pointing to molecular mechanisms underlying this process. Ongoing studies of mutants and drug assays in both systems will help to elucidate parasite factors involved in *Leishmania* quiescence and increase understanding of the role of quiescent parasites in persistence and treatment failure.

Keywords QUIESCENCE; PERSISTENCE; DRUG TREATMENT FAILURE; HOST-PARASITE RESPONSE; OMIC TECHNOLOGIES

O33-04: PROTEOMICS-BASED INVESTIGATION OF SUBSTRATES FOR THE *Leishmania* DEUBIQUITINASE DUB2

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The deubiquitinating enzyme (DUB)-mediated cleavage of ubiquitin plays a critical role in balancing protein synthesis and degradation. Twenty DUBs exist in the *Leishmania mexicana* parasite, of which four, including DUB2, are essential for the viability of *L. mexicana* promastigotes. DUB2 has a broad ubiquitin linkage specificity, and it is known to be crucial in establishing infection in mice. However, the functional role of DUB2 is not clear. Thus, we aim to identify the substrates of DUB2 through a comprehensive proteome, ubiquitinome and interactome analysis using mass-spectrometry-based quantitative proteomics, affinity-based ubiquitinated peptide enrichment and proximity dependent biotinylation. For the latter approach, 84 proximal proteins to DUB2 were identified as being significantly enriched. Gene ontology enrichment analysis categorised these proximal proteins to 17 biological processes, with protein translation being the most significant, followed by RNA binding/processing, and microtubule-associated functions, suggesting that DUB2 might have a pleiotropic function. Furthermore, initial investigation of the total ubiquitinome in *L. mexicana* using a Data Dependent Acquisition (DDA) mass-spectrometry workflow revealed that 28 of the DUB2 proximal proteins are ubiquitinated, suggesting that these might be substrates of DUB2. Currently, we are investigating whether some of these proximal proteins are interacting partners of DUB2 via co-immunoprecipitation and we are characterising the total ubiquitinome of *L. mexicana* via an improved proteomics Data Independent Acquisition (DIA) methodology.

Keywords: *Leishmania mexicana;* DEUBIQUITINASE; PROTEOMICS; UBIQUITOMICS; PROXIMITY LABELLING

O33-05: EXPLOITING THE PROTIST *Leishmania tarentolae* TO GENERATE A CANDIDATE VACCINE AGAINST COVID-19

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As an alternative platform for vaccine production, we exploited the protozoan *Leishmania tarentolae*, as a microfactory to produce antigens of the SARS-CoV-2 virus, the etiological agent of COVID-19. *L. tarentolae* is a protozoan parasite not pathogenic to humans, classified as a biosafety level class I organism and already developed as system to produce recombinant mammalian proteins, due to the protein glycosylation pattern guaranteed by this microbe, which mimics that of vertebrates. Moreover, *Leishmania* parasites have a natural tendency to penetrate inside dendritic cells (DCs), the cells that trigger and govern the immune response. These parasites are also expected to induce strong antibody responses. Therefore, to obtain a candidate vaccine against COVID-19, we engineered two strains of *L. tarentolae* for the expression of antigens from the SARS-CoV-2 virus: a first strain produces the whole Spike protein of the virus, exposing it on its surface; a second strain secretes a fragment of the Spike protein into the culture medium; this fragment is thus suitable for an effective purification. The two components (the strains expressing the Spike protein at its surface and the purified protein fragment) were then combined to obtain the

candidate LeCoVax2 vaccine. LeCoVax2 was first tested in *in vitro* assays on myeloid cells (macrophages and DCs); it was then tested *in vivo* in rodent models, assaying various routes of administration. *In vitro* assays proved that LeCoVax2 was able to stimulate the maturation of human DCs and to induce a mild immune modulation toward a Th1-promoting phenotype. The experiments carried out on the mouse model, after parenteral delivery of the antigen, demonstrated that LeCoVax2 is capable to determine specific and neutralizing antibody responses, with no evidence for undesired effects in mice. Furthermore, we focused on the mucosal route for the administration of LeCoVax2; the obtained results showed that the vaccine determined the production of antibodies after rectal delivery, including IgAs, which are potentially involved in the blocking of mucosal colonization by the virus, at the first phases of infection. In conclusion, our study confirms the potential of *L. tarentolae* as a scaffold for the production and delivery of viral antigens, to be used as vaccines. More specifically, antigens of SARS-CoV-2, administered through the LeCoVax2 preparation, determined an effective antibody response.

Keywords SARS-CoV-2, VIRAL ANTIGEN EXPRESSION, EXPRESSION SYSTEMS, EPIDEMICS

Financing Erogazione liberale per le attività di ricerca sul Coronavirus"; "Fondazione Romeo ed Enrica Invernizzi

O33-06: COMPARATIVE ANALYSIS OF THE MICROBIOME ASSOCIATED WITH CUTANEOUS LESIONS CAUSED BY *Leishmania braziliensis* USING BIOINFORMATICS TECHNIQUES

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Tegumentary leishmaniasis (TL) is a globally distributed human disease characterized by a spectrum of clinical manifestations, ranging from self-healing single lesions to chronic and metastatic lesions. An exaggerated immune response leading to excessive inflammation is associated with the aggravation of the disease and can be influenced, among other factors, by the symbiotic relationship between *Leishmania RNA Virus* 1 (LRV1) and *Leishmania* spp. Skin microbial communities have been shown to be essential for skin health and immune function. Indeed, dysbiosis of the skin microbiome has been linked to several inflammatory diseases, including TL. We conducted skin microbiome analysis, based on sequencing the 16S rRNA, of patients presenting TL. Comparison between non-injured skin and cutaneous lesions were performed, including leishmaniasis and non-leishmaniasis patients. Several analyses were performed, including species richness, species diversity, bacterial community composition, and differential abundance between set of samples. The time of evolution of the lesions, environmental factors and the presence/absence of LRV1 were considered. Bacterial diversity in healthy skin was higher than in cutaneous leishmaniasis lesions. The microbial communities differed between samples from leishmaniasis and non-leishmaniasis patients in both non-injured skin and lesions. It was also observed that the longevity of Leishmania-infected

lesions impacts microbial community diversity. As hypothesized, the presence of LRV1 impacts microbial community diversity and samples from patients presenting LRV1-Leishmania infection show differences in bacterial abundance and composition. Noteworthy was the similarity observed in the bacterial species diversity between cutaneous and mucosal lesions, but microbial composition differed accordingly to clinical manifestation. This study suggests that microbial communities on cutaneous leishmaniasis lesion are distinct from those observed in other types of lesions clinically confused with leishmaniasis and reinforce the idea of a microbiome profile associated to cutaneous leishmaniasis, characterized by lower bacteria diversity in lesions than in healthy skin and by microbial dysbiosis portrayed by the high predominance of *Staphylococcus* in any skin site. The severe immune response present in mucosal lesions would explain the differences observed in microbial composition between mucosal and cutaneous lesions. Exacerbate immune response is also often associated with LRV1-infected *Leishmania* which can explain the influence of LRV1 in the microbiome already impacted by *Leishmania* infection.

Keywords *Leishmania braziliensis;* LEISHMANIA RNA VIRUS 1, LRV, MICROBIOME

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4.7 SOCIAL INNOVATION - IMPLEMENTATION RESEARCH - OPERATIVE RESEARCH

08-01: ACCEPTABILITY AND MICRO-COSTING OF MICROSCOPY AND RAPID DIAGNOSTIC TEST OF LOCALISED CUTANEOUS LEISHMANIASIS IN MOROCCO: AN EXPLANATORY MIXED STUDY

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In Morocco, Cutaneous Leishmaniasis (CL) is due to three species *L.tropica, L.major, L.infantum.* Up to date, no CL rapid diagnostic test (RDT) is included in any country control strategy. Recently, an innovative quick diagnostic test for CL (CL Detect™ Rapid Test) was developed by Inbios® International Seattle, USA and assessed in some countries. This study aimed to explain the acceptability and costs of this test, from health professionals' perspective, in case of being proposed as a new CL management tool in Moroccan health facilities. Between June 2019 and January 2020, self-administered questionnaires and face to face in-depth individual interviews were used with 46 health professionals working in 40 selected primary health centres (PHCs) and district laboratories. For this explanatory mixed-methods study, including a micro-costing and a qualitative data analysis, all participants

were invited by the investigators to see at the beginning a video demonstrating how to perform CL RDT before gathering information about the CL diagnostic pathway and their expected costs for both diagnostic tools (microscopy and CL RDT). The qualitative information was categorized initially by using one sheet of paper. After that, a computer-assisted qualitative data analysis, type NVivo software, allowed cross-matched qualitative results with the quantitative ones. The data triangulation helped to foster the understanding. The thematic analysis allowed developing an explanation of what was going on in the data by capturing different dimensions to present the main finding: The health professionals acceptability's compared to their previous CL RDTs' use or knowledge, and the micro-costing of CL RDT compared to microscopy. November 2019 rate change was used, US$1=9.6MAD (Moroccan dirham). CL RDT test was appreciated by almost all Health Professionals enrolled at the PHCs and laboratories. Who expressed its advantage compared to the microscopy diagnosis, qualifying the CL RDT as a new tool easily, safely, and quickly used for CL confirmation diagnostic at the first visit at the PHC, done in 29 minutes [12min - 45min]. The HP previous users of CL RDT formulated the strongest quotes about its usefulness. Moreover, the overall mean cost of CL RDT was US$10.7 [8.5-14.8] (purchased unitary strip at US$6.4) compared to the overall mean cost of US$11.8 [6.1-30.4] for microscopy. As almost 75% of CL RDT cost is linked to the CL RDT strip price, the micro-costing advantage is reached if the strip will be purchased not more than US$5.6 (54MAD). In contrast, the microscopy could be advantageous if the purchased CL RDT strip would be higher than US$7.6 (73MAD). As a future new CL management tool in Moroccan health facilities, CL RDT is widely accepted and less costly than microscopy. Otherwise, private drug stores' support can sustain this CL RDT diagnostic activity in endemic CL areas with scarce human qualified resources.

Keywords CUTANEOUS LEISHMANIASIS; POINT OF CARE; MICROSCOPY; COSTS; ACCEPTABILITY.

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O8-02: QUALITY OF LIFE OF CUTANEOUS LEISHMANIASIS SUSPECTED PATIENTS IN THE ECUADORIAN AMAZON AND PACIFIC REGIONS: A CROSS-SECTIONAL STUDY

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Cutaneous Leishmaniasis (CL) affects an estimated 5000 patients yearly in Ecuador with clusters in the Amazon and subtropical Pacific regions. CL leads to reduced Health Related Quality of Life (HRQL) as a result of social and self-stigma in the Asian and Mediterranean contexts. In contrast, the few studies that assessed HRQL of CL patients in northern South America found no evidence for stigmatization but research is lacking for Ecuador. This study explores the influence of CL suspected lesions on the quality of life of patients in the Amazon and subtropical Pacific regions. Patients were included in private and public primary health care centers and hospitals in the Amazonian Napo, Pastaza, and Morona Santiago provinces and in the Pacific region of the Pichincha province. Participating centers offered free of charge CL treatment. All patients suspected of CL and subsequently referred

for a cutaneous smear slide microscopy examination were eligible. Patients were included from January 2019 through June 2021. Patients who answered less than 75% of the questionnaire items were excluded. This study applied the Skindex-29 questionnaire, a generic tool to measure HRQL in patients with skin diseases. The questionnaire contains 29 questions related to three dimensions: Emotional, Symptoms, and Functioning. The Amazonian and Pacific regions are divided by the Ecuadorian highlands where leishmaniasis is rare. The prevalent *Leishmania* species, vector-human interaction, and social structure in the two regions differ and were therefore analyzed separately. All statistical analysis was done with SPSS Statistics version 28. Mean Skindex-29 scores were assessed for statistical significance with the independent samples t-test or one-way ANOVA. The skindex-29 questionnaire was completed adequately by 279 patients who were included in this study. All ethnic patient groups from the Amazon scored significantly ($P < 0,01$) higher (indicating worse HRQL) on all the dimensions of the Skindex-29 questionnaire than Mestizo patients from the Pacific region. The present study revealed that the influence of suspected CL lesions on the HRQL of patients in the Ecuadorian Amazon and subtropical Pacific depends on the geographic region, more than on patient characteristics such as gender, age, number of lesions, lesion type, location of lesions, health-seeking delay, or posterior confirmation of the *Leishmania* parasite. The percentage of patients with a health-seeking delay of less than a month was significantly ($P < 0,01$) lower in the Amazon region (38%) than in the Pacific (66%). The impaired HRQL in Amazonian patients might be the result of stigma expressions as found during qualitative interviews. The health-seeking delay might result from mobility barriers due to hinterland circumstances, lack of recourses, discrimination, failing diagnostic tests, and/or stigmatization as occurs with leprosy patients. Together, the impaired HRQL and health-seeking delay lead to prolonged suffering and a worse health outcome. The lack of Skindex-29 questionnaire validation in Amerindian and <18 populations limits this study. Determinants of health-seeking delay should be clarified in future studies of health-seeking behaviors and CL case finding must be improved by the health authorities. Moreover, HRQL analysis in other CL endemic regions could improve local health management.

Keywords LEISHMANIASIS, CUTANEOUS; QUALITY OF LIFE; TIME-TO-TREATMENT; GEOGRAPHIC LOCATIONS; ECUADOR

O8-03: EMPOWERING PEOPLE WITH CUTANEOUS LEISHMANIASIS: AN INTRODUCTION TO THE ECLIPSE PROGRAMME

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In this presentation I will introduce the interdisciplinary ECLIPSE programme and its aims. ECLIPSE is a four-year £4.6M healthcare programme funded by the UK's National Institute for Health Research (NIHR), which aims to improve the CL patient journey and reduce stigma in the most underserved communities in Brazil, Ethiopia and Sri Lanka. ECLIPSE brings together leishmaniasis expertise in an international, cross-cultural, multidisciplinary team of over 60 researchers, including anthropologists, parasitologists, clinicians from different medical specialties, psychologists, disease specialist and public health researchers. The ECLIPSE team are working towards a patient journey that is holistic, patient-centred and mapped on a biopsychosocial model of CL. Two interventions will be co-developed, implemented and evaluated in each ECLIPSE country, aimed at promoting early diagnosis and treatment seeking behaviour, decreasing social isolation and stigma, empowering CL-endemic communities and improving treatment pathways. The ECLIPSE team are using a range of qualitative and quantitative methods and anthropological theories to gain in-depth understanding of people, communities and healthcare professionals experiences and views on the effects of CL on the daily lives of those affected, the barriers to seeking healthcare, obtaining accurate, early diagnosis and receiving effective treatment. The insights gained will inform the development of new interventions: community education campaigns to increase disease awareness and reduce stigma and training packages for healthcare professionals. The ECLIPSE team is strongly committed to involving community members in all the ECLIPSE

activities in partner countries. This means that each stage of our applied health programme is conducted with community members, in line with our ethos: '*no research about us, without us*'. We recognise, value and wish to amplify the community knowledge and understandings of health and illness, and the facilitators and challenges to seeking treatment for CL. This recognition places community engagement and involvement at the heart of ECLIPSE. The communities' experiential knowledge, combined with other knowledge (such as biomedical and anthropological insights), will result in the co-creation of new knowledge which will underpin the ECLIPSE interventions.

Keywords CUTANEOUS LEISHMANIASIS; STIGMA; SOCIAL SCIENCES; ANTHROPOLOGY; INTERVENTIONS

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O8-04: "EVEN IF HE GETS HUNGRY OR THIRSTY, HE'LL ENDURE ALL JUST FOR THE SAKE OF TREATMENT COST": EXPLORING THE ECONOMIC IMPACT OF CUTANEOUS LEISHMANIASIS AND LEPROSY ON HOUSEHOLDS IN RURAL ETHIOPIA

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Cutaneous leishmaniasis and leprosy are stigmatizing skin diseases often resulting in substantial morbidity and disability. The economic burden of these skin diseases has not been well documented. The aim of this qualitative study was to explore the household economic impact of cutaneous leishmaniasis and leprosy in rural Ethiopia and the strategies used by affected households to cope with this economic burden. The study was conducted in Kalu district, South Wollo Zone, Amhara Region of Ethiopia from March to June 2021. It forms one part of multidisciplinary formative research conducted to support development of an integrated intervention strategy appropriate to the local context by Skin Health Africa Research Programme (SHARP). Qualitative data collection explored both the economic questions presented here, and also other topics, including stigma and disease discourses. In-depth interviews (n=98) with patients, caregivers, and health workers; focus group discussions (n=40) with community members; and key informant interviews (n=50) with opinion leaders, traditional healers and policy actors were conducted. Data were coded using MAXQDA 2020 software and thematic framework was used for analysis. Individuals with cutaneous leishmaniasis (CL) and leprosy and their family members experienced high cost for seeking care in the form of out-of-pocket payments, especially for transport and accommodation. Experiencing these illnesses resulted in loss of income to the household,

through wage loss to both patients and other household members, notably accompanying persons in care-seeking. The findings show that children with CL and leprosy were sometimes absent from school or withdrew from school entirely because of their conditions. Several coping strategies including asset selling, consumption reduction, contracting out land to be farmed, borrowing, family and community support and community-based health insurance were used by the patient and family members to mitigate the financial costs of illness and production losses. Nonetheless, strategies like consumption reduction and selling assets are more common among leprosy patients and their families. Households in which an individual experiences CL or leprosy face substantial economic impact in terms of lost income and time for care-seeking. Strengthening treatment and diagnostic facilities closer to communities may increase access and reduce transport and travel costs. Including transportation costs within financial risk protection mechanisms may alleviate the financial impact.
Keywords CUTANEOUS LEISHMANIASIS; LEPROSY; ECONOMIC IMPACT; QUALITATIVE STUDY; HOUSEHOLD COSTS

O8-05: MEASURING AWARENESS AND STIGMA OF CUTANEOUS LEISHMANIASIS: A PILOT STUDY IN ENDEMIC RURAL UNDERSERVED COMMUNITIES OF THE SOUTH OF BAHIA, BRAZIL

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The knowledge and awareness on cutaneous leishmaniasis (CL) are considered fundamental to improve prevention habits and healthy behaviours in the local contexts. Moreover, these 2 dimensions can be strongly associated with CL stigma. Consequently, the CL awareness and stigma measurement are necessary actions to evaluate the relevance and the impacts of health education interventions. This study is part of a 4 years international and multisite research project ECLIPSE - Empowering people with Cutaneous Leishmaniasis: Intervention Programme to improve patient journey and reduce Stigma via community Education – focused on community engagement and involvement against CL, and it is realized in three countries - Sri Lanka, Ethiopia and Brazil, coordinated by UK's academic research institutions. We present here the results of the pilot study of CL awareness and stigma measurement we have conducted with patients and healthcare professionals of CL hyperendemic rural areas in the South of Bahia, Brazil. This preliminary study was realized in order to evaluate the quality of the questionnaire we will apply in the ECLIPSE research sites (~450 persons) during 2022. The first version of the questionnaire was at first adapted using the findings from the qualitative research – 75 qualitative interviews focusing on the CL experience

conducted in July 2021. Secondly, we find necessary to model this instrument respecting a cultural appropriated language, and we discuss with the community members group of the ECLIPSE project – community leaders, local health and education workers, professionals, managers and politicians – and also with CL Brazilian experts participating to the ECLIPSE project – during 2021. Finally, we applied the questionnaire with 50 participants of 2 training courses focusing on CL ecology – February/march 2022 – and realized a discussion after its use to check eventual misunderstanding of the questions and the evaluation of its quality. The participant´s profile was mainly characterized by the presence of black woman (89,2%), with high educational levels (64,3%), only health professionals – mainly nurses, 1 physician and 10 community health workers. Even if most of the participants did not have direct experience of CL (81,7%), most of them knew CL (92,2%) or knew someone with CL experience (88,90%). However, the CL awareness level was very low, particularly on the causes and transmission (45% had no idea or was not able to correctly identify the vector), or indicated "junky places" as the origin of the proliferation (24%). Most of the participants think CL can kill (72,2%), and generalist answers were given about the drugs and biomedical treatment practices. On the contrary, the stigma scale pointed to high levels of stigma for the CL patients. In the participants perception, CL patients use to hide their scars (83,4%), they suffer shame or embarrassment (72,2%), are avoided by community members (64,4%), have problems to stay or find a work (65,7%) or to find a partner (78,1%). After this piloting research action, we find that the questionnaire is ready to be used within the rural communities and it is useful to measure these 2 central dimensions of the experience of CL.

Keywords STIGMA; AWARENESS; RURAL COMMUNITY; QUESTIONNAIRE; CUTANEOUS LEISHMANIASIS; BRAZIL

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O8-06: SOCIAL SCIENCES AS A KEY ASPECT FOR THE UNDERSTANDING AND PREVENTION OF CUTANEOUS LEISHMANIASIS IN RURAL COMMUNITIES IN COLOMBIA

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Community engagement in biomedical research is key to gain public recognition of its social value and improve implementation and uptake of research findings. CIDEIM has been integrating Social Sciences in its biomedical research portfolio on cutaneous leishmaniasis (CL), to contribute to the community uptake of knowledge as a strategy to promote a cultural sensitive understanding of the disease, as well as the importance of a timely diagnosis, and potential community actions for prevention and control of CL in specific contexts. Two social science approaches have been integrated in CIDEIM´s biomedical research projects: 1) Participatory-Action Research (PAR), a methodology that involves researchers and communities in a self-reflective process to understand social problems and take action to solve them; and 2) Social Appropriation of Knowledge (SAK), understood as a process that promotes dialogue and exchange of knowledge and experiences between different actors, including communities, in order to make scientific knowledge and technology available to the population, so they can apply it to their own needs. These projects are being developed in 3 endemic areas in Colombia: Pueblo Rico-Risaralda; Tumaco-Nariño and Rovira-Tolima; municipalities inhabited by ethnic minorities in rural areas who have limited access to health services due to geographic and socio-cultural conditions. Three of our projects illustrate these efforts: A first project (2019-2020) that brought together researchers and community

leaders from Tumaco, Rovira and Pueblo Rico, in a co-creation process for the production of radio capsules and educational materials for control of CL, as a strategy for its proper transmission and use among their own communities. A second project (2021) that aimed to implement a community surveillance model, to improve the detection of cases of CL in Pueblo Rico (Risaralda), through participatory and playful activities. This initiative, in addition to promoting collaborative efforts among community leaders, health workers and researchers; strengthened leadership skills; and resulted in community engagement products such as songs, stories, podcasts, flyers and Tiktoks, with context-specific content associated to CL and other infectious diseases. Finally, a public engagement project (2021) aiming to raise awareness about the importance of timely diagnosis, prevention and treatment of CL in rural communities of Tumaco, developed "Zumbidos en el Río", a socially-contextualized radio drama co-created with local health workers, community leaders and researchers. Our experience has allowed the promotion of collaborative innovation spaces, including researchers, communities and other stakeholders in the development of concepts, products and solutions in response to health problems in specific contexts. It has also shown that these co-creative activities as well as the participation of communities in the different phases of research, enable empowerment of the community and knowledge uptake in order to create positive change. However, measuring impact of these efforts remains a challenge. The integration of PAR and SAK approaches in biomedical research projects may facilitate community access and use of scientific knowledge and social transformation in health.

Keywords SOCIAL SCIENCES; CUTANEOUS LEISHMANIASIS; PREVENTION; PARTICIPATION; COMMUNITY

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O16-01: COMMUNITY PARTICIPATION IN THE FIGHT AGAINST LEISHMANIASIS

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Leishmaniasis is a parasitic disease, constitutes a public health problem. Its control requires the mutual support of the different stakeholders: health professionals and the community. This is a descriptive exploratory study to determine status of community participation in the control of leishmaniasis in an endemic area "Province of Al Haouz" of Marrakech Safi region. A questionnaire was sent to health professionals in Al Haouz province (n=50). In addition to an interview with the population of Al Haouz over than 18 years (n=384) to explore knowledge, attitudes and practices. The main results revealed that health professionals' knowledge of leishmaniasis is moderate to three types of leishmaniasis, diagnosis, nature of the disease, and dominant form in the province. The knowledge about the transmission of disease, reservoir, vector species, actions of GILAV committee is low. The knowledge acquired about the disease comes mainly from experience or in some cases from initial training. However, there is a lack of training of health professionals in relation to leishmaniasis in Al Haouz. Finally, no one reported participation in a community-based project to control leishmaniasis. The interviewed population is dominated by women (56%), cutaneous leishmaniasis is the only form known by 28% of the population. Respondents' knowledge about leishmaniasis is low regarding transmission, causes, types and preventive measures of leishmaniasis. In

addition, the population uses traditional remedies to drive out the vector or treat skin leishmaniasis, no leishmaniasis prevention materials from the local authorities have been distributed, nor have education and awareness sessions on leishmaniasis been organized. The gap in knowledge, attitudes and practices of the population regarding leishmaniasis, and the low level of involvement of health professionals in the activities of the leishmaniasis control program, justify the absence of community participation.

Keywords LEISHMANIASIS; PREVENTION; PARTICIPATORY APPROACH; ATTITUDE; KNOWLEDGE; PRACTICE; COMMUNITY

O16-02: EFFECTIVENESS AND ACCEPTABILITY OF COMMUNITY TREATMENT FOR CUTANEOUS LEISHMANIASIS IN ETHIOPIA - A MULTI-DISCIPLINARY STUDY

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Cutaneous leishmaniasis (CL) is a neglected tropical disease transmitted by sandflies which poses a large public health problem in Ochollo village, southern Ethiopia. Besides hyraxes as animal reservoirs, humans are fueling transmission of *Leishmania aethiopica* too. Therefore, early diagnosis and treatment of CL patients are pivotal to reduce the human reservoir and CL transmission in the area. There is very little knowledge about treatment effectiveness in African CL patients, especially for community-based treatment, and how this affects transmission. It is also crucial to assess community perspectives on modern CL treatment in order to understand patient needs, to optimize interventions, and to ensure maximized utility of generated evidence for policy makers. In this study, we aimed to investigate the acceptability and effectiveness of community-based treatment and its impact on disease transmission. A multi-disciplinary study is being carried out from October 2021 to August 2022 in Ochollo. Community leaders and health extension workers were engaged to actively search for suspected CL patients and send them to the health post. They were included if clinically diagnosed as CL by a physician or if they were microscopy positive. Patients were assigned to systemic miltefosine or localized cryotherapy treatment depending on their lesion characteristics. Cryotherapy was given weekly for

4 weeks, and miltefosine daily for 28 days, using allometric dosing for children. Clinical, patient-reported, and parasitological outcomes are assessed at one, three, and six months after the start of treatment. Cure is defined as complete flattening and/or reepithelization six months after the start of treatment. Patient questionnaires and interviews with patients, previous patients, health workers, traditional healers and community leaders were done to assess acceptability of modern community-based treatment. These will be repeated after treatment to assess changing perspectives. To assess whether treatment of human cases can reduce transmission, *Leishmania* prevalence is determined in sandflies indoors in suspected CL houses and in caves before and after the intervention in Ochollo and a control village where no treatment is given to see whether the prevalence decreases in the intervention village. The entomological study was initiated in October 2021, and the acceptability assessment and treatment of patients was started in January 2022. We included 108 patients, of which 43 were assigned to miltefosine, 48 to cryotherapy and 17 were not treated. At the conference, we will present the CL lesions that occur in the community, and show preliminary data of the effectiveness of cryotherapy and miltefosine at one and three months after treatment, using clinical, patient-reported and parasitological outcomes. We will discuss the perception and acceptability towards modern treatment both before and after the intervention, to assess whether such interventions are feasible for scale-up. Findings from this project will yield important information on the acceptability and effectiveness of cryotherapy and miltefosine treatment in a community-setting. We will also be able to draw conclusions on the potential impact of such a community-based treatment intervention on the overall disease transmission. This information will be vital to move towards large-scale studies for treatment and control of CL in Ethiopia.

Keywords TRANSMISSION; *Leishmania aethiopica*; COMMUNITY-BASED TREATMENT; INTERVENTION; PERSPECTIVES

O16-03: THERAPEUTIC ITINERARIES OF PEOPLE WITH CUTANEOUS LEISHMANIASIS IN RURAL SETTINGS IN BAHIA: COMMUNITY CARE STRATEGIES AND THE CHALLENGES TO ACCESS HEALTH CARE

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This paper aims to discuss the therapeutic itineraries of people with Cutaneous Leishmaniasis (CL), focusing on community care strategies and health practices within and outside the formal health system. CL is a neglected disease, hyperendemic in certain regions of Bahia, which affects mainly farming families in rural areas. The paper emerges from the partial results of ECLIPSE Project - Empowering people with Cutaneous Leishmaniasis (CL): Research and Intervention Program to improve patient itineraries and reduce stigma through community engagement. This is a multicenter, qualitative-based, interdisciplinary and participatory project led by Keele University (UK) and developed in communities in Brazil (FASA/ISC/UFBA; SIM/HUPES/UFBA), Ethiopia and Sri Lanka. In Brazil, the project covers rural communities in the municipalities of Valença, Tancredo Neves and Teolândia, recognized as endemic areas for CL in Bahia. The data was produced through semi-structured interviews, as well as conversation circles and other community-based participatory methodologies. The data analysis allowed us to conclude that, when identifying skin lesions, families seek the reference service for CL in Corte de Pedra and adhere to medical treatment. However, they report suffering experiences both with the disease and with the drug treatment, which is usually prolonged and not very adaptable to the life contexts of these communities. The importance of Community Health Agents in the identification of the disease, referral to formal health services, and adherence to treatment can be observed. Families' previous experiences and accumulated knowledge also contribute to the therapeutic itineraries. Cultural knowledge and practices of care are

also observed in these communities, although little recognized and valued by the hegemonic biomedical model. Therapeutic itineraries can also include hospitalizations, interfering in family arrangements and complexifying the treatment experience. Barriers to access to care are also observed, especially geographic and climatic barriers, also including financial difficulties that impact the mobility of service users. It is imperative to recognize that the experience of illness from CL is crossed by markers of class, race, gender, generation, and physical and/or mental disabilities that become determinants in the social production of the disease and reproduction of health inequities.

Keywords THERAPEUTIC; ITINERARY; LEISHMANIASIS; RURAL; HEALTH

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O16-04: PILOT PROGRAM FOR COMMUNITY AND INSTITUTIONAL STRENGTHENING ON CUTANEOUS LEISHMANIASIS IN ENDEMIC AREAS MUNICIPALITIES

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The challenge of recognizing and treating vector-borne diseases is faced in dispersed rural communities and health services at the basic level. On Cutaneous Leishmaniasis (CL), particularly, gaps in knowledge as well as in the comprehensive care of the disease are present. A set of interventions has been designed taking into account the concepts of Citizen Science and Research Implementation, aimed at strengthening presumptive diagnosis, early detection, recognition of the disease into the community context, diagnostic confirmation, comprehensive care, and monitoring of therapeutic response. These actions have been developed by a group of previously trained professionals (an anthropologist, a communicator and some community agents) who carry out activities in the villages with the communities. Similarly, another group (a microbiologist and a doctor) works in the strengthening of parasitological diagnosis, therapeutic options, and follow-up of therapeutic response, both in first and second level hospitals. In the community context, the starting point was the characterization of risks, behaviors and knowledge about CL. This allowed the design of health educational strategies such as: 1) Periodic workshops with health commissions on diagnosis and treatment, vector behavior, and CL preventive measures. 2) Training leaders in presumptive diagnosis for the early detection of injuries and early referral to health institutions, based on a presumptive diagnosis file. 3) Transmedial production of communicative pieces: podcasts, radio soap operas, radio spots, illustrated stories, murals and songs. For health institutions in 10 municipalities, a diagnosis was made of the epidemiological behavior of CL and about

knowledge gaps into the health teams on the national guidelines for the comprehensive care of patients with CL. Discussion and training spaces were created on the epidemiology of CL, presumptive diagnosis, confirmatory diagnosis, therapeutic options, and follow-up of therapeutic response of CL, with quarterly follow-up to these interventions. Early detection of cases in the community has increased by 30%, and the sensitivity of health teams has improved access to quality confirmatory diagnosis and the use of better tolerated therapeutic options. It has assured a better adherence, and an early detection of failure in the treatment, from constant monitoring in the field. Based on the concepts of Citizen Science and and Research Implementation, coordinated actions between the community and health teams work in processes of identifying facilitators and limitations. This community strategy can allow better results in the response to neglected and difficult-to-treat diseases. This pilot test is expected to raise awareness in communities and health teams about the prevention and control of CL, and to offer new elements to research teams. Articulating the work with the communities around the knowledge of the behavior of diseases transmitted by vectors can allow the prevention of diseases, the early detection of risks and symptoms, and enable timely attention to the cases.

Keywords CUTANEOUS LEISHMANIASIS; PRESUMPTIVE DIAGNOSIS; CITIZEN SCIENCE; RESEARCH IMPLEMENTATION

Financing National School of Public Health, Universidad de Antioquia, Epidemiological Surveillance System

O16-05: INVOLVING PATIENTS IN DRUG DEVELOPMENT FOR NTDS: A QUALITATIVE STUDY EXPLORING PATIENTS' PREFERENCES TO INFORM TARGET PRODUCT PROFILES (TPPS) FOR CUTANEOUS LEISHMANIASIS

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Target Product Profiles (TPPs) for therapeutics, vaccines, and diagnostics are a valuable instrument to optimise product design and development. In public health, they are important for the final product to be aligned with priorities and users' needs, and to achieve the intended impact. There is limited guidance on how to construct TPPs, and methodologies vary. While the World Health Organization has recently published guidelines for developing TPPs, there are few examples of patients' involvement as stakeholders in building a TPP. This exploratory study presents a methodological and analytical approach on how to engage with patients on aspects related to the attributes of a new drug, with a focus on Cutaneous

Leishmaniasis (CL), and how this could inform TPPs for NTDs. Thirty-five cutaneous leishmaniasis (CL) patients from Brazil, Colombia, and Austria were recruited using a maximum variation approach along geographic, sociodemographic and clinical criteria. Semi-structured in-depth interviews were conducted in the patients' mother tongues, using a comprehensive interview topic guide asking about disease experiences, preferences and expectations. Transcripts, translated into English, were analysed using a qualitative framework analysis approach. The TPP developed by DND*i* (Drug for Neglected Diseases initiative) specifically for CL treatment was the initial coding framework. We were able to match disease experiences, preferences, and expectations of a broad spectrum of CL patients to a TPP framework addressing the categories safety, efficacy, costs, treatment schedule (including route of administration) and perceived barriers. Patients' preferences regarding treatments ranged from specific efficacy endpoints to direct and significant indirect costs they would need to meet, e.g., costs associated with inability to work, or relocation for the duration of treatment. Often, elements were assessed against each other in relation to efficacy and experienced discomfort/adverse events, e.g., where a more painful infection would be accepted if it led to a shorter duration and was perceived to clear parasites quickly. Patients also discussed many actual and potential reasons for non-compliance, such as experienced adverse events or geographical and availability barriers. Resources for drug development for NTDs are very limited. NTDs affect disadvantaged populations often living in challenging conditions with little or no access to health systems. Engaging patients in designing adapted therapies could significantly contribute to the suitability of an intervention to a specific context and to compliance, by tailoring the product to the end-users' needs. This could include using safety and efficacy endpoints important to patients in trials, or improving compliance by product design. Qualitative methods can also identify trade-offs that users are willing to accept, such as parenteral route of administration if the product reduces number of days of treatment or monitoring requirements. This exploratory study identified preferences in a broad international patient spectrum, and provides methodological guidance on how patients can be meaningfully involved as stakeholders in the construction of a TPP for a drug to treat an NTD, and thus be actively involved in drug development.

Keywords CUTANEOUS LEISHMANIASIS; DRUG DEVELOPMENT; QUALITATIVE; TARGET PRODUCT PROFILE

O16-06: STUDY OF IMPACT OF IRRIGATION WATER MANAGEMENT ON THE INCIDENCE OF LEISHMANIASIS - CASE OF ENDEMIC FOCI IN JERADA PROVINCE

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Irrigation water management is usually evaluated by considering technical, economic and environmental performance indicators. However, few studies have focused on the impact on human health. The literature review on this subject showed a positive correlation between the extension of irrigated areas and the prevalence of cutaneous zoonotic leishmaniasis (CZL). Indeed, irrigation practices increase soil moisture and subsequently provide suitable ecological niches for the development of vectors (sandflies) and reservoirs (rodents) of cutaneous leishmaniasis. The present work reports the results of retrospective and prospective ecological epidemiological study of the vector (sandfly) and reservoir (rodent) of the endemic foci of "Ain Bni Mathar", "Bni Mathar" and "Ouled Sidi Abdelhakem" of Jerada province between 2010 and 2016 and its relationship with the expansion of irrigation practices. The endemic foci studied total 76% of cases (223 cases) of the province of Jerada, with predominantly young (70% of cases do not exceed 40 years) and from 82% of the rural area with spatial concentration of cases of LZC in the peri-urban area of Ain Bni Mathar. The mammalogical study of rodents identified *Meriones shawi* as a reservoir mainly in the cereal crop fields bordering the infested foci. These foci are strictly associated with state-supported irrigable agricultural development areas, thus facilitating the attraction and colonization of *Meriones shawi* reservoirs of cutaneous zoonotic leishmaniasis (CZL). It is therefore recommended that health impact studies of agricultural development projects be conducted,

particularly before starting irrigation expansion projects, to prevent further expansion of ZLB.

4.8 VECTORS & RESERVOIRS

023-01: GENETIC DIVERSITY AND HAPLOTYPE ANALYSIS OF *Leishmania tropica* IDENTIFIED IN SAND FLY VECTORS OF THE GENERA *Phlebotomus* AND *Sergentomyia*, USING NEXT-GENERATION-SEQUENCING TECHNOLOGY

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Leishmaniases are vector borne-diseases caused by a variety of Leishmania parasite species with clinical picture ranging from mild cutaneous leishmaniasis (CL) to fatal visceral leishmaniasis (VL). In this study, next-generation sequencing (NGS) was used to determine the infection of *Leishmania tropica* in the sandfly vector and investigate the genetic diversity the same *Leishmania spp*. A two-step multiplex PCR was utilized in detecting *Leishmania* DNA in the sand-fly vector using ITS1NGSF- ITS1NGSR primer pair targeting the internal transcribed spacer 1 (ITS1) of the genus *Leishmania,* and SFNGSF and SFNGSR primer pair used to detect sand-fly DNA targeting a specific fragment in the 18S rRNA gene of Phlebotominae subfamily. Amplified DNA was deeply sequenced using the Nextseq500 Illumina technology. Bioinformatics analyses were conducted using Galaxy, MEGA version X, DnaSP ver. 6.12.03, and PopArt 1.7 software for NGS, phylogenetic tree, genetic diversity, and haplotype networking, respectively. A total of 307 engorged sandflies were trapped from hyrax dens with an overall *Leishmania* infection rate of 9.4% (29/307) and 6.8% by NGS and ITS1-PCR, respectively. Two sandfly genera were trapped,

Phlebotomus and *Sergentomyia* with earlier having higher rate of *Leishmania* infection (10.2%, 26/254) than later (5.7%, 3/54). Of the P*hlebotomus* spp., 44% (136/307) were *P. sergenti.* The phylogenetic tree showed two bootstrap-supported clusters, a major cluster (cluster I) that included the four study sequences along with 22 Genbank-retrieved DNA sequences. A minor cluster (Cluster II) consisted of three sequences from Iran and Pakistan. The genetic diversity analysis for the 29 *L. tropica* sequences showed high haplotype (gene) diversity index (Hd) (0.62± 0.07), but low nucleotide diversity index (π) (0.04± 0.01). DnaSP ver. 6.12.03 estimated the total number of haplotypes for clusters I and II at 8 and 3, respectively. Tajima's D, a neutrality test, is more negative in cluster I (D=-2.0) than in total population, (D=-1.83), but both are equally significant (P<0.001) indicting that observed variation in cluster I and whole population is less frequent than expected. The median-joining haplotype network constructed by PopArt 1.7 produced a total of 11 active haplotypes. In conclusion, *L. tropica* from sand flies in Palestine is monophyletic that assembled in one main phylogroup and one haplotype. The study revealed one high genetically diverse population (cluster II) and one relatively low genetically diverse population (cluster I) regardless of origin of samples, geographic or isolation.

Key words: GENETIC DIVERSITY; HAPLOTYPE NETWORK ANALYSIS; NEXT-GENERATION SEQUENCING; PALESTINE; *Leishmania*

O23-02: CUTANEOUS LEISHMANIASIS INCUBATION PERIOD IN FRENCH GUIANA IS SHORTER THAN EXPECTED: IMPLICATIONS FOR SEASONALITY

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The cutaneous leishmaniasis (CL) incubation period (IP) is defined as the time between parasite inoculation by sandfly bite and the onset of the first CL skin lesion. This period is not clearly established and few studies have tried to estimate it. Military personnel in French Guiana (FG) are prospectively followed with great accuracy, as part of the epidemiological surveillance system in French armed forces. They come from non-endemic metropolitan France and stay in FG for few weeks or months. Therefore, this

population is appropriate to determine the average CL IP. We retrospectively studied the anonymized data from military personnel with a confirmed diagnosis of CL between January 2001 and December 2021, after a short stay in FG. Dates of arrival in FG, departure from FG, and onset of symptoms were available. A diagnosis of CL was confirmed in case of compatible lesions and at least one positive test among smear, parasite culture or PCR. The CL IP distribution was estimated using time-to-event models adapted to interval-censored data. A total of 180 patients were included. Almost all of them were men (177/180, 98.3%), and median age was 26.2 (23.1-31.2) years. When recorded, the parasite species was always *Leishmania guyanensis* (30/180, 16.7%). The most frequent place of contamination was the Center for Equatorial Forest Training on the Eastern coastal region (82/112, 73.2%). The main periods of CL diagnosis spread from November to January (84/180, 46.7%) and in March-April (54/180, 30.0%). The IP was estimated at 25–26 days (95% CI, 20–30 days) using a non-parametric method. This IP estimation was 25.9 days (95% CI, 23.5–28.5 days) when using Bayesian AFT regression. The IP was 7.8 days (95% CI, 6.2–9.8 days) in 5% of cases and did not exceed 61.5 days (95% CI, 55.7–69.3 days) in 95% of cases, according to Bayesian AFT regression. This study provides a rare insight of CL IP. Indeed, it is not easy to determine the precise date of contamination in endemic areas because they occur year-round and are rarely recorded due to the absence of prospective cohorts. In FG, climatic time series have suggested that patients were infected during the late dry season (August to October) and seeked care for their lesions around January, which suggested an IP of several months. Our study suggests that IP is probably shorter and close to the results of Aoun et al. who reported a median IP of 5 weeks after infective trips to an endemic area in Tunisia. Therefore, the predominance of CL diagnosis in the first months of the year in FG may be explained by contaminations during the short rainy season (November-January) and at the beginning of the long rainy season (March-April). It is possible that the occurrence of short dry periods would play a role in expanding phlebotomine populations. This work suggests that the CL IP is shorter than expected, about one month. This finding implies that contaminations probably occur during rainy seasons and not in the late dry season as previously reported.

Keywords CUTANEOUS LEISHMANIASIS; INCUBATION; FRENCH GUIANA; MILITARY; CLIMATE

O23-03: OVIPOSITION ECOLOGY OF *Phlebotomus papatasi* SAND FLIES: PATTERNS, PROCESSES, AND APPLICATIONS

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A sustainable alternative to the delivery of an insecticide to the vector is to bring the vector to the insecticide using attractants. In this project, we applied a multi-disciplinary approach for bioassay-guided fractionation of semiochemicals from organic matter and conspecific origin for developing an optimal oviposition lure for the control and surveillance of *Phlebotomus papatasi* sand flies (a vector of Old-World cutaneous leishmaniasis [CL]). We identified larval conditioned rearing medium as a potent source for oviposition attractants and stimulants. By evaluating the bacterial composition and gravid sand flies' attraction to larval conditioned and medium aged under the same conditions but without larvae, we showed that sand flies' attractiveness to larval conditioned medium over aged medium is associated with a differential community compositions of the two rearing substates. We isolated and cultured 12 bacterial strains from the most attractive medium type (rearing medium containing 2nd-3rd instar larvae) and showed that gravid female's attraction was mediated by a sub-set of highly attractive bacterial strains that produce several volatile attractive compounds. We also screened various conspecific stages as a potential source of oviposition attractants. We found that conspecific eggs and first instar larvae were highly attractive and stimulated oviposition whereas older stages were not. Conspecific eggs had a dose dependent effect on sand fly behavior inducing attraction at low-intermediate doses and repellence at high doses. We found that this attraction pattern was mediated by dodecanoic acid as an egg and larval pheromone. We also identified

Isovaleric acid as an important attractant produced by both young conspecific stages as well as by the most attractive bacterial isolate. In addition to these olfactory-driven processes, we discovered that attraction to potential oviposition sites is mediated by visual cues with gravid females, but not any other stage, attracted to dark oviposition cups, probably, resembling host rodent burrows. Furthermore, we noticed that when gravid females were presented with a black oviposition cup the attractive olfactory effect of organic matter was diminished. Finally, our study also described the circadian rhythm of *P. papatasi* oviposition behavior, with gravid females increasing their affinity to volatile oviposition attractants at the later part of the night whereas actual egg-laying tended to mostly occur at the earlier part of the night. These findings are instrumental in guiding us through the effort of developing an attractive lure that could be used for the surveillance of sand flies as well as serve as a centerpiece in a lethal oviposition trap that could be used to trap gravid *P. papatasi* females and thereby reduce the transmission of *L. major* in endemic CL regions. Furthermore, this approach could be expanded to other sand fly systems, thereby adding a novel tool for combatting Leishmaniasis around the world

Keywords VECTOR-HOST COUPLING; BIOLOGICAL CONTROL; DISEASE ECOLOGY; OVIPOSITION ATTRACTANTS; CUTANEOUS LEISHMANIASIS

23-04: DOMESTIC MAMMALS AS POTENTIAL RESERVOIR HOSTS FOR *Leishmania donovani* IN INDIA

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Visceral Leishmaniasis on the Indian subcontinent is thought to have an anthroponotic transmission cycle. There is no direct evidence that a mammalian host other than humans can be infected with *Leishmania donovani* and transmit infection to the sand fly vector. Recent studies of the potential impact of sand fly feeding on other domestic species in the Indian subcontinent found evidence of sand fly feeding on dogs, cows, water buffaloes and goats, which begs the question: "Is there a possibility of non-human reservoirs?" In order to answer this critical question, we collected blood from these animals for qPCR and serology and performed xenodiagnosis using colonized *Phlebotomus argentipes* sand flies to feed on animals residing in villages with active *Leishmania* transmission based on current human cases. Xenodiagnoses on animals within the endemic area were performed and blood-fed flies were analyzed for the presence of *Leishmania* via qPCR 48hrs after feeding. We found positive evidence of *Leishmania* infection in these domestic mammals but they were not infectious to vector sand flies. Monitoring infection in sand flies and non-human blood meal sources in endemic villages leads to scientific proof of exposure and parasitemia in resident animals. Lack of infectiousness of these domestic animals to vector sand flies indicates that they likely play no role, or a very limited role in *Leishmania donovani* transmission to people in Bihar. Therefore, a surveillance system in the peri-/post-elimination phase of visceral leishmaniasis (VL) must monitor absence of transmission.

Continued surveillance of domestic animals in outbreak villages is necessary to ensure that a non-human reservoir is not established, including animals not present in this study, dogs.

Keywords DOMESTIC ANIMALS; VISCERAL LEISHMANIASIS; ZOONOSES; *Phlebotomus argentipes*; INDIA

O23-05: *Leishmania* GENOMIC ADAPTATION DURING EXPERIMENTAL SAND FLY INFECTION

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Trypanosomatid pathogens are transmitted by blood-feeding insects, causing devastating human infections. Parasite survival in these vertebrate and invertebrate hosts relies on the differentiation of distinct developmental stages that are the result of a long-term, co-evolutionary process. These stages show in addition important phenotypic plasticity that often impacts infection, affecting for example parasite pathogenicity, tissue tropism, or drug susceptibility. Despite their clinical relevance, the short-term evolutionary mechanisms that allow for the selection of such adaptive phenotypes remains only poorly investigated. Here we use *Leishmania donovani* as a trypanosomatid model pathogen to shed first light on parasite evolutionary adaptation during experimental sand fly infection. Applying a comparative genomic approach on hamster-isolated amastigotes and derived promastigotes before (input) and after (output) infection of *Phlebotomus orientalis* confirmed a strong bottle neck effect as judged by principal component and phylogenetic analysis of input and output parasite DNA sequences. Despite the bottle neck effect, our analyses revealed various genomic signals that seem under positive selection given their convergence between independent, biological replicates. While no significant fluctuations in gene copy number were revealed between input and output parasites, convergent selection was observed for karyotype, haplotype and allelic changes during sand fly infection. Our analyses further identified signature mutations of oxidative DNA damage in the output parasite

genomes, suggesting that *Leishmania* suffers from oxidative stress inside the insect digestive tract. Our data call into question previous observations on *Leishmania* resistance to reactive oxygen species and propose ROS-induced DNA repair processes as drivers of haplotype and allelic selection during sand fly infection. The experimental and computational framework presented here provides a useful blueprint to assess the evolutionary adaptation of other eukaryotic pathogens inside their insect vectors, such as *Plasmodium* spp, *Trypanosoma brucei* and *Trypanosoma cruzi*.

Keywords *Leishmania donovani*; EXPERIMENTAL SAND FLY INFECTION; GENOME SEQUENCING; GENOMIC ADAPTATION; ALLELIC SELECTION

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O23-06: PRESENCE OF *Lutzomyia cruzi* (DIPTERA; PSYCHODIDAE) IN A NEW TRANSMISSION FOCUS OF VISCERAL LEISHMANIASIS IN VILLAMONTES, TARIJA, BOLIVIA

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In December 2021, the first two cases of human visceral leishmaniasis (VL) were reported in two children from the city of Villamontes in Tarija, becoming the first reported and confirmed cases of VL in an area with no prior transmission. Of these, the second case diagnosed by the Entomology and Parasitology Laboratory at the National Institute of Health Laboratories (INLASA), through a rapid test (rk39), observation of amastigotes in bone marrow aspirate smears, and conventional PCR in a bone marrow aspirate sample. For this reason, INLASA in coordination with the National Programme of Vector Borne Diseases of the Ministry of Health, the Local Health Service (SEDES – Tarija), the Coordination of Health Networks of Villamontes and the Center of Tropical Diseases (CENETROP), carried out a focus study with the objective of identifying the presence of vector species at the probable site of infection, applying the methodology described in the "Leishmaniasis in the Americas Surveillance Manual" of the Pan-American Health Organization (PAHO). Phlebotomines sandflies were captured in three neighborhoods of Villamontes, two of them located in the peri-urban area of the city. For the captures, CDC light traps were installed in the intra-domiciliary, peri domicile and extra domicile area of three houses in each neighborhood. A total of 276 sandflies were captured, 69 non-fed females, 62 engorged females and 145 males. The engorged females were saved for further natural infection and food source studies, thus, 214 sandflies captured in intra, peri and extra domicile were identified. The collected sandflies correspond to the following neighborhoods of Villamontes: 1. Ferroviario Bajo, where the second case of VL was presented; 2. Villa Esperanza, where the first human case was diagnosed, and 3. Marzana. In

Ferroviario Bajo neighborhood, 156 sandflies of the following species were identified; *Evandromyia cortelezzii, Lutzomyia cruzi, Migonemyia migonei, Micropygomyia sp*. and *Nyssomyia sp.* In Villa Esperanza, 49 sandflies were identified; *Ev. cortelezzii, Psathyromyia sp., Mi. migonei* and *Lu. cruzi*, and in the Marzana neighborhood 8 sandflies; *Lu. cruzi* and *Trichophoromyia* sp. The dominant captured species were *Mi. migonei* (63 %), followed by *Lu. cruzi* (26%) captured both indoors and outdoors, *Ev. cortelezzii* (12%) and to a lesser extent, *Micropygomyia sp., Psathiromyia sp.* and *Nyssomyia* sp. The identification of *Lu. cruzi* together with the diagnosis of the first cases, confirmed the transmission of Visceral Leishmaniasis in Villamontes. However, the presence and possible epidemiological role of *Mi. migonei* cannot be excluded. It is important to continue and deepen the research with emphasis on the identification of circulating *Leishmania* as the causal agent of VL in canine reservoirs and humans, as well as the natural infection of phlebotomines sandflies.

Keywords *Lutzomyia cruzi*; VISCERAL LEISHMANIASIS; NEW FOCUS; INLASA

O30-01: THE EFFECT OF THE SUGAR METABOLISM ON *Leishmania infantum* PROMASTIGOTES INSIDE THE GUT OF *Lutzomyia longipalpis*: A SWEET RELATIONSHIP?

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It is well-known that *Leishmania* parasites can alter the behavior of the sand fly vector in order to increase their transmission potential. However, little is known about the contribution of the infecting host's blood composition on subsequent sand fly infection and survival. This study focused on the host's glucose metabolism and the insulin/insulin-like growth factor 1 (IGF-1) pathway as both metabolic processes are known to impact vector-parasite interactions of other protozoa and insect species. The focus of this study was inspired by the observation that the glycemic levels in the blood of infected Syrian golden hamsters inversely correlated to splenic and hepatic parasite burdens. To evaluate the biological impact of these findings on further transmission, *Lutzomyia longipalpis* sand flies were infected with blood that was artificially supplemented with different physiological concentrations of several monosaccharides, insulin or IGF-1. Normoglycemic levels resulted in transiently higher parasite loads and faster appearance of metacyclics, whereas higher carbohydrate and insulin/IGF-1 levels favored sand fly survival. Although the recorded effects were rather modest or transient of nature, these observations support the concept that the host blood biochemistry may affect *Leishmania* transmission and sand fly longevity.

Keywords GLUCOSE; INSULIN; SAND FLY; *Leishmania*

O30-02: DRUG RESISTANCE AND TREATMENT FAILURE IN VISCERAL LEISHMANIASIS: WHAT CAN WE LEARN FROM SAND FLY AND RODENT INFECTIONS?

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Therapeutic failure in visceral leishmaniasis (VL) patients is known to have a multifactorial origin involving drug, host and parasite related factors. As drug resistance or phenotypic adaptations of *Leishmania* leading to less effective treatment pose a significant threat to control programs in disease endemic countries, the risk of propagation of such traits needs to be carefully considered. There are also major knowledge gaps in host related aspects of therapeutic failure that represent an important constraint in the development of long-term effective drugs. This presentation will cover various research lines at the University of Antwerp that address major outstanding questions about resistance propagation and efficacy of treatment. The establishment of a *Lutzomyia longipalpis* sand fly colony in a new insectarium and the in-house generation of engineered parasite strains, enabled us to study the impact of drug resistance on natural transmission by the insect vector and infection of the vertebrate host. The underlying mechanisms of resistance were found to determine the phenotypic traits and capacity to be effectively transmitted and establish infections. While for some drugs rapid adaptation of parasites to monotherapy and spread of the acquired resistance is a substantial risk, other resistance-conferring mutations were found to result in severe attenuation. *In vivo* bioluminescent mouse infection models also pointed to niches where parasites can survive treatment, seeding the recolonization of the host. Large-scale *in vivo* drug screening and immunophenotyping identified hematopoietic stem cells with exceptionally high parasite burdens that are more tolerant to antileishmanial drug action. Various molecular and immunological analyses have been applied to understand

Leishmania persistence in these stem cells. The presented work reveals some of the bottlenecks in the sand fly and vertebrate host that affect treatment efficacy and the propagation of resistance. Besides recommendations for rational drug use, insights in molecular mechanisms of parasite persistence may open new therapeutic avenues for leishmaniasis.

Keywords VISCERAL LEISHMANIASIS; RESISTANCE; TRANSMISSION; TREATMENT FAILURE

O30-03: ENTOMOLOGICAL DRIVERS OF *Leishmania* TRANSMISSION IN VILLAGES WITH AND WITHOUT VISCERAL LEISHMANIASIS CASES IN ENDEMIC DISTRICTS OF EASTERN NEPAL

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Visceral leishmaniasis (VL) or kala-azar is caused by *Leishmania donovani* and transmitted through the bite of the female vector sand fly *Phlebotomus argentipes* in Nepal. The country has been committed to eliminate the disease as a public health problem since 2005, with a target annual incidence below one per 10,000 population at district level. Although Nepal was the first country to achieve this target in 2013, the national VL elimination programme is still confronted with many challenges like the increasingly wide-spread distribution of VL cases over the country, resurgence and the questionable efficacy of the main vector control activity i.e., indoor residual spraying (IRS). In this study, we aimed to generate contemporary data on entomological factors to provide sound recommendations to address these challenges. We collected information on entomological drivers like seasonality in vector density, prevalence of *Leishmania* infection in the vector and host preferences which are crucial for evidence-based adaptation of effective vector control measures during the peri elimination era. The data were collected in two epidemiologically contrasting settings i.e., villages where VL cases were reported over the last three years (VL village) and nearby village without such reported cases

(non-VL village) over last ten years within the three VL endemic districts; Morang, Sunsari and Saptari in eastern part of Nepal. Adult sand flies were collected using CDC light traps and mouth aspirator in each village for 12 consecutive months from July 2017 to June 2018. Sand fly species identification was done morphologically and verified by DNA barcoding. *Leishamania* infection was assessed in gravid sand flies specimens targeting the 115 bp length of small-subunit ribosomal RNA gene of *Leishmania* (SSU-rRNA). Vertebrate cytochrome b (*cyt b*) gene of ~ 350 bp segment was amplified in blood-fed sand flies collected from the dwellings shared by both human and cattle to identify preferred host. A total of 39,692 Phlebotomine sand flies were captured and *P. argentipes* (79.06%; 31,382) was the predominant species. Higher number of *P. argentipes* was captured in non-VL villages (n=21,743; <0.001) compared to VL villages. Seasonality in *P. argentipes* sand fly density was observed with peaks in July and September in VL villages and June, July and November in non-VL villages. Estimated prevalence of *Leishmania* infection rate in vector sand flies was found to be 2.2% (1.1% — 3.7% at 95% credible interval) in VL villages and 0.6% (0.2% — 1.2% at 95% credible interval) in non-VL villages. The most common source of the blood meal was human beings with 52.7% in VL villages and 74.1% in non-VL villages followed by cattle. High vector density, anthropophilic nature and the detection of *Leishmania* infection in vector sand flies in both type of epidemiological settings indicated the ongoing leishmania transmission in endemic districts and relaxation of vector control program would have deteriorating consequences. In this context, vector control measures should be continued in the VL reported and surrounding areas with suitable adaptation in timing accompanied with intensive disease and vector surveillance in assurance to sustain the VL elimination in Nepal.

Keywords VISCERAL LEISHMANIASIS; VECTOR CONTROL; SEASONALITY; *Leishmania* INFECTION; BLOOD MEAL

O30-04: BLOOD FEEDING SOURCES OF *Nyssomyia antunesi*, A SUSPECTED VECTOR OF *Leishmania* IN THE BRAZILIAN AMAZON

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The zoonotic nature of leishmaniases places the investigation of potential reservoirs in the priority list of surveillance strategies. Thus, identifying phlebotomine blood meal sources can be an alternative way to improve knowledge on this subject. Present work aimed to identify blood feeding sources of *Nyssomyia antunesi* females, a suspected vector of *Leishmania* sp., from an urban park in the urban center of Belém city, the capital of Pará State, in the Brazilian Amazon. *Leishmania* DNA detection was also attempted. Entire bodies and gut contents of *Ny. antunesi* engorged females, previously captured in the urban park of Belém city, Brazil, with CDC light traps and aspiration on tree bases, were submitted to *Leishmania* and vertebrate DNA detection through amplification of the leishmanian mini-exon and vertebrate cytochrome b (cyt b) gene regions, respectively. Quality of DNA extraction from entire bodies was ensured through amplification of a dipteran cytb region. The vertebrate cyt b amplicons were sequenced and compared with those available on GenBank. A maximum likelihood phylogenetic tree was reconstructed to assess the clustering pattern of these sequences. No *Leishmania* DNA was detected. Sequences of 13 vertebrate cytb amplicons were considered informative and phylogenetically

supported to exhibit similarity/clustering with the following six vertebrate species: *Dasyprocta leporina* (1), *Cuniculus paca* (1), *Tamandua tetradactyla* (4), *Choloepus didactylus* (4), *Pteroglossus aracari aracari* (2), and *Homo sapiens* (1). The samples of *D. leporina* and *C. paca* were from CDC canopy, while the others were from aspiration on tree bases. Present results revealed an eclectic and opportunist blood feeding behavior of *Ny. antunesi*, including birds and mammals, these last ones acting as potential reservoirs of *Leishmania* species, distributed throughout the vertical forest strata.

Keywords PHLEBOTOMINAE; HOST; MAMMAL; TRANSMISSION

Financing IEC/MS; NMT/UFPA

O30-05: KNOCKDOWN RESISTANCE MUTATIONS IN *Phlebotomus argentipes* FROM VILLAGES WITH AND WITHOUT INDOOR RESIDUAL SPRAYING IN BIHAR, INDIA

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Vector control by indoor residual spraying (IRS) is one of the main components of the visceral leishmaniasis (VL) elimination programme in India. DDT was used for IRS until 2015, then later replaced by the pyrethroid alpha-cypermethrin. DDT and alpha-cypermethrin both target the voltage gated sodium channel (*vgsc*) and high levels of resistance to DDT have been documented in the local sand fly vector, *Phlebotomus argentipes.* The aim was to compare the frequency of knockdown resistance (kdr) mutations in *vgsc*, particularly at codon 1014, observed in two sprayed and two unsprayed villages in Bihar state, India, which has the highest VL burden of the four endemic states. *Phlebotomus argentipes* were collected in 2019 in four villages in Bihar during a molecular xenomonitoring study: two VL endemic villages receiving IRS and two villages with no IRS. 350 females were used for sequence analysis of the IIS6 fragment of the *vgsc* gene and the frequency of mutations at codon 1014 was compared between sprayed and unsprayed villages. A high frequency of *kdr* mutations was found in the study. Mutations were identified at various positions within the IIS6 fragment, most frequently at codon 1014. Significant inter-village variation was observed. Sand flies from Dharampur, a non-sprayed village, had a significantly higher proportion of wild type alleles (55.8%) compared with the three other villages (8.5 – 14.3%). Both L1014S and L1014F mutations were observed, but the frequency of mutations where amino acid serine replaced leucine (L1014S) was the highest (48.5%). Significant differences in the frequencies of mutations observed between the villages may have

occurred as a result of selection pressure caused by previous exposure to insecticides. *Kdr* mutations L1014S and L1014F are associated with insecticide resistance in many vectors, including sand flies. While DDT resistance has been reported in Bihar, *P. argentipes* is still susceptible to pyrethroids. However, the presence of *kdr* in sand flies could present a threat to IRS used for VL control in endemic villages in India so the use of different insecticide classes, such as carbamates, organophosphates or neonicotinoids, may be explored for the purpose of insecticide resistance management. Further research on vector bionomics and insecticide resistance will be required to inform India's vector control strategies and ensure the VL elimination target is reached. This may include pairing of bioassay data to confirm the resistance phenotype of sand fly populations with genotypic data and transcription levels of metabolic enzymes that cause resistance to insecticides.

Keywords VISCERAL LEISHMANIASIS; VECTOR SURVEILLANCE; INSECTICIDE RESISTANCE; MOLECULAR XENOMONITORING

Funding The Bill and Melinda Gates Foundation supported the study through the SPEAK India consortium (OPP1183986)

O30-06: CHARACTERIZATION OF THE MICROBIOTA IN *Lutzomyia longipalpis* (DIPTERA PSYCHODIDAE) SUBJECTED TO A TEMPERATURE GRADIENT DEVICE

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Cases of leishmaniasis have increased in the last decade as climate change exerts pressure over insects that need to adapt as well as the pathogens they carry, favoring the spread of vector-borne diseases in new places. In nature, small fluctuations in microclimatic conditions can have a profound effect on the insects, however, most studies focus on laboratory populations and macro-climatic data from the area of influence. It is also known that the microbiota of insect vectors varies with environmental changes; its participation in immune response, fitness, and resistance to insecticides could influence the transmission rate of human pathogens, as is the case of *Leishmania* parasites. Analyzing temperature preferences in phlebotomine sand flies and the characterization of the temperature effects on the abundance and richness of the total microbiota of *Lutzomyia longipalpis*, the insect vector of *Leishmania infantum* in America, is a relevant aspect to understand how temperature shifts can affect the potential transmission of *Leishmania*. We developed and used a customized device with a temperature gradient (21-34°C) to expose wild females of *Lu. longipalpis* collected in a rural area of Ricaurte, Cundinamarca, as well as females of *Pintomyia evansi*, that was used as a control and was collected in a peri-urban area of Sincelejo, Sucre, in Colombia. A total of 7 and 5 replicas were conducted with 50 females each, who were exposed to such a gradient for

an hour. In the end, individuals of *Lu. longipalpis* were collected and classified by temperature ranges between 21°C and 34 °C, resulting in 17 pools. Additionally, total DNA extracts were obtained and samples were subjected to 16S amplicon sequencing analyses. We found that both species preferred temperatures between 21 - 23 °C with an average of 23 females of *Lu. longipalpis* and 20 females of *Pi. evansi*, with significant differences in the behavior presented by both sand flies ($p<0.05$) as the latter showed higher abundances in warmer environments between 25°C - 31 °C. Microbiota characterization of *Lu. longipalpis* showed that the most abundant phyla were Proteobacteria (90.26%) and Firmicutes (73.52%). Results also show an abundance of 57.36% in *Pseudomonas* genus at 25-27 °C that decreases to 6.55% at 29-31°C and 13.20% at 31-33°C (p-value < 0.055), while at the same ranges *Bacillus* presents an abundance of 1.21%, 61.54% and 37.64% respectively (p-value < 0.096). No significant differences were found when comparing richness indices, while β-diversity differences were found especially at 29-33 °C (p-value < 0.013). It was also possible to detect *Spiroplasma, Rickettsia,* and *Asaia* bacteria infection, but with low prevalence, while infection of *Arsenophonus* was also present with positive interactions with *Bacillus* (PCC 0.77, p-value of 0.006, and FDR of 0.015). The results of this study show that there is a variation in microbiota abundances and changes in bacterial communities of *Lu. longipalpis* considering the temperature preferences of the insect. However, more studies should be performed to further elucidate the implication of such changes in sand fly microbiota.

Keywords TEMPERATURE PREFERENCES; ENDOSYMBIONT; MICROBIOTA; *Lutzomyia longipalpis*

O25-06: THE ROLE OF DOG AS POTENTIAL RESERVOIR HOST OF *Leishmania infantum* AND TOSCANA VIRUS IN A ZOONOTIC VISCERAL LEISHMANIASIS FOCUS OF NORTHERN TUNISIA

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Phlebotomus perniciosus is the main vector of *Leishmania infantum*, etiological agent of zoonotic visceral leishmaniasis (ZVL) affecting humans and dogs in the Western Mediterranean basin. It is well known that *P. perniciosus* is the main vector of Toscana virus (TOSV), a sandfly-borne phlebovirus that exhibits neurotropism and may lead to aseptic meningitis and meningoencephalitis in the Mediterranean area. While dog is the main reservoir of *L. infantum*, its role as reservoir host for Toscana virus remains unanswered. This study investigated *L. infantum* and TOSV infection in dogs with canine leishmaniasis (CL) following natural exposition during the season of sandfly's activity from June to October 2020 in a ZVL focus located in Northern Tunisia. Concomitantly, infection prevalence of sandflies with *L. infantum* and TOSV was studied during the same period of dog's exposition in the same site. A total of three dogs (A, B, C) with CL and one healthy dog were tested by xenodiagnosis using a colony of *P. perniciosus*. Pools of freshly engorged *P. perniciosus* were screened for TOSV and *L. infantum* by nested PCR in the polymerase gene and kinetoplast minicircle DNA, respectively. The same experiment was performed with 5-days fed *P. perniciosus* and with field-collected sandflies. *L. infantum* DNA and TOSV RNA were detected in females *P. perniciosus* fed on dog B and C, respectively, No pathogens were detected in females *P. perniciosus* fed on dog A and control. Pathogens screening performed on 6211 field-collected sandflies

distributed in 223 pools revealed the presence of TOSV RNA and *L. infantum*. The minimum infection rates of sandflies with TOSV and *L. infantum* were 0.09%, and 0. 037 %, respectively. Our results provide strong evidence that in ZVL endemic foci where both pathogens are co-circulating, dogs get infected with *L. infantum* and with TOSV and transmit the infection to sandfly vector populations. Therefore, dogs are considered as potential reservoir hosts for TOSV in addition to its role as the main reservoir of *L. infantum*. More studies are needed to better understad the role of TOSV as a potential co-factor in the development of ZVL.

Keywords *Leishmania infantum;* TOSCANA VIRUS; *Phlebotomus perniciosus*; DOGS; RESERVOIR

5. POSTER

5.1 CANINE LEISHMANIASIS

P1-002: PREVALENCE AND RISK FACTORS OF *Leishmania* INFECTION IN DOGS IN PORTUGAL- A CROSS-SECTIONAL STUDY

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Canine leishmaniosis (CanL) caused by *Leishmania infantum* is an important zoonosis in southern European countries where this disease is endemic and dogs, as domestic animals, are in close contact with humans. In Portugal CanL assumes a relevant veterinary concern. The last national seroprevalence study was conducted over a decade ago, in 2009, with an overall seroprevalence of 6.3%. Since then, new prophylactic measures, such as vaccines, have been introduced in Europe. The aim of this study was to update seroprevalence for *Leishmania* infection and re-access risk factors in Portugal. A cross-sectional study was conducted in Jan-Mar 2021 in domestic dogs from mainland Portugal with the participation of 98 Veterinary clinics. A questionnaire (dogs' age, sex, breed, living habits, prophylactic measures, and presence of clinical signs compatible with CanL) and canine samples (whole blood on filter paper) were collected with informed consent from dog owners. Direct agglutination test (DAT, cut-off titer=400) was used to calculate anti-*Leishmania* antibody titres. Quantitative and qualitative variables and serological results were analysed by IBM® SPSS® Statistics Version 2.0 to perform descriptive statistics, univariate and multivariate analysis. Through multiple binary logistic regression models, the adjusted odds ratio values (aOR) were estimated, and some associated risk factors were identified. The true seroprevalence was estimated on Epitools© Epidemiological Calculators considering 100% test specificity and 93% test sensitivity. A total of 1860 dogs were screened and 34.8% (n= 648/1860) of

dogs lived mostly or exclusively outdoors. The use of effective repellent insecticides was reported in 41.5% (774/1860) of dogs and 14.9% (271/1860) were vaccinated. True seroprevalence in the country was 12.5% (95%CI 10.3 - 13.2%), varying from 30.5% (95%CI 19.9 - 43.8) to 0.0% (95%CI 0.0 - 7.5) in the different Districts, reflecting an increase when compared with the 2009 study. Pure breeds presented higher positivity than mongrel dogs (12.9%, 119/923 vs 10.5%, 98/937). No differences were observed concerning fur size. A higher percentage of positive dogs was observed in dogs not using repellent/insecticides (13.1%, 73/556 vs 12.2%, 90/772). Positivity in vaccinated dogs was 26.5% (72/271) but dogs with Canileish® presented higher positivity (47.6%, 30/63) than Letifend® (18.3%, 26/142). Among dogs presenting clinical signs, 37.5% (42/112) were positive. Only 6.9% (5/72) of vaccinated positive dogs presented clinical signs, compared to 26% (37/142) non-vaccinated positive dogs with clinical signs, demonstrating the major role of vaccination in the prevention of the disease in endemic areas. Risk factors associated with *L. infantum* infection in dogs were age-dogs with ≥ 2 years (aOR = 2.14, 95%CI 1.45 - 3.14), and residing in the interior regions of the country (aOR =1.63, 95%CI 0.91 – 1.72). The non-use of repellents/insecticides was also identified as a risk factor (aOR = 1.74, 95%CI 1.20 – 2.53) in non-vaccinated dogs. The key to control CanL and its impact on Public Health in Portugal but also in other endemic areas of Europe lies on continuous implementation of prophylactic measures, through the correct use of repellents/ insecticides and vaccines, early detection and monitoring of infected dogs.

Keywords CANINE LEISHMANIOSIS; PORTUGAL; DAT; RISK FACTORS; *Leishmania*

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P1-003: METALLOPROTEINASES 2 AND 9 DETECTION IN THE AQUEOUS HUMOR OF DOGS WITH LEISHMANIASIS

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Ocular and periocular manifestations in canine visceral leishmaniasis (CanL) is a frequent lesion and usually are associated with other systemic findings of this disease. Local granulomatous inflammation and/or the production of immune complexes may be involved in the pathogenesis of ocular lesions. MMPs are capable of degrading almost all components of ECM and thus play important roles in many physiological and pathological processes including some affections of the eye. As far as we know there are no reports on the participation of MMPs in the pathogenesis of eye lesions in dogs with CanL. Aqueous humor of 28 animals diagnosed with visceral leishmaniasis were used in this study, considering parasitological (FNA) and serological (ELISA) parameters. Samples from three healthy animals were used to compose the control group. The search for inactive and active forms of MMP-2 and MMP-9 was performed using aqueous humor zymography. We performed histopathological examination of 21 eyes of infected dogs. Mononuclear inflammatory infiltrate was found in all 21 samples most commonly in the limbus (n=17) and ciliary body (n=14), followed by perineurium of optical nerve (n=12) and the iris (n=10). Anti-leishmania antibodies were detected in the aqueous humor of infected dogs (OD > 0.270). Zymography revealed the presence of the active form of MMP-2 in all samples (n =31) from dogs with leishmaniasis and also from the control group, while proMMP-2 was not detected in any sample. Regarding MMP-9, its two forms were found in 7 dogs out of 28 positives for leishmaniasis. The inactive form of MMP-2 is constitutive in the aqueous humor. The inactive and active forms of MMP-9 have already been

associated with corneal regeneration, uveitis and keratoconjunctivitis sicca, but it is not described in healthy eyes. MMP9 is a known mediator of inflammation and plays a role in tissue repair. The presence of the active form of MMP-9 in the aqueous humor of dogs with leishmaniasis suggests a role for this MMP in the pathogenesis of eye lesions described in the dogs.

Keywords INFLAMMATION; UVEITIS; ZYMOGRAPHY; EYE

Financing The project was in part supported by FAPESP# 2016/02384-9, CAPES, CNPq-PIBIC.

P1-005: COMPARISON OF FOUR COMMERCIAL SEROLOGICAL TESTS FOR THE DETECTION OF *Leishmania infantum* INFECTION IN DOGS

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Early detection and treatment of cases of *Leishmania infantum* infestation are considered to be critical in controlling the spread of the disease in dogs; besides this, it is also considered to be very important to reduce the zoonotic threat of the disease. Various serological methods are available to support the diagnosis of canine leishmaniosis: immunofluorescence antibody test (IFA), enzyme-linked immunosorbent assay (ELISA), immunochromatography test, direct agglutination test, western blot, and latex agglutination test. Several quantitative serological tests are commercially available; however, their effectiveness can vary widely and this, therefore, affects the ability to reach a correct diagnosis in dogs. High antibody levels are associated with severe parasitism and disease and are diagnostic of clinical leishmaniosis. In contrast, the presence of low antibody levels is not necessarily indicative of disease and it might be more difficult for it to be detected by serological tests. It is interesting to note that a high proportion of seropositive dogs would be considered to be apparently healthy. The aim of this study was to evaluate and compare the diagnostic performance of four commercially available serological tests including ELISAs (CIVTEST® CANIS LEISHMANIA short and standard protocol, LEISHMANIA-ELISA DOG®, ELISA/S7®) and MegaFLUO®LEISH IFA for the detection of specific antibodies against *L. infantum* antigens in dogs in different infective states. Canine sera samples from sick infected seropositive dogs (n=37), seropositive apparently healthy dogs (n = 29), and

apparently healthy seronegative dogs from endemic (n=32) and non-endemic areas (n= 40) were classified based on the results of an in-house UAB ELISA. All canine sera samples were analysed using the commercial serological methods mentioned above following the manufacturers' recommendations. The sensitivity and specificity observed for each test was as follows: CIVTEST® standard (90.4%, 86%), CIVTEST® short (81%, 90%), LEISHMANIA-ELISA DOG® (82.7%, 64%), ELISA/S7® (32.7%, 26.7%) and MegaFLUO®LEISH IFA (100%, 83.7%), respectively. The accuracy was as follows: CIVTEST® standard (0.88), CIVTEST® short (0.86), LEISHMANIA-ELISA DOG® (0.71), ELISA/S7® (0.29), and MegaFLUO®LEISH IFA (0.90). The Kappa index from best to worst was: MegaFLUO®LEISH IFA (K= 0.80), CIVTEST® standard (K= 0.75), CIVTEST® short (K= 0.71), LEISHMANIA-ELISA DOG® (K=0.43), ELISA/S7® (K= -0.37). The Youden index was: MegaFLUO®LEISH IFA 0.84, CIVTEST® standard 0.76, CIVTEST® short 0.70, LEISHMANIA-ELISA DOG® 0.47%, ELISA/S7® -0.41%. Finally, the area under the ROC curve (AUC-ROC), ordered from the maximum to the minimum value was: CIVTEST® standard (0.94), CIVTEST short ® (0.93), LEISHMANIA-ELISA DOG® (0.82), and ELISA/S7® (0.25). In summary, the results showed that CIVTEST® CANIS LEISHMANIA performed better than the rest of the commercial ELISA assays that were tested, regardless of the type of protocol used. Notably, ELISA/S7® showed the most controversial results; further research is needed to investigate this lack of agreement and the implication for the diagnosis of the disease. In conclusion, this study confirmed that the performances of the commercially available ELISA tests against *L. infantum* can vary widely. Moreover, it highlights the fact that CIVTEST® CANIS LEISHMANIA is a reliable test to support the diagnosis of canine leishmaniosis in clinical settings.

Keywords ANTIBODY; CANINE; DIAGNOSIS; ELISA; LEISHMANIOSIS

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P1-006: PREDICTIVE FACTORS FOR CANINE VISCERAL LEISHMANIASIS IN FOUR ENDEMIC MUNICIPALITIES IN BRAZIL

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The objective was to develop and validate a clinical-laboratory prediction model for *Leishmania infantum* infection in dogs in Brazil, associating results of the dual-path platform chromatographic immunoassay (DPP® CVL rapid test) and clinical signs of the disease. We used secondary data on clinical and laboratory information from 1600 adult dogs of both sexes, and different breeds, generated by a cross-sectional, multicenter, population-based study carried out from October 2008 to April 2009 in four municipalities endemic for canine visceral leishmaniasis (CVL) in Brazil (Brasília/DF, Palmas/TO, Bauru/SP and Fortaleza/CE). The predictor variables were age, 14 clinical signs, and the DPP-CVL result, while the outcome variable was CVL, defined through parasitological tests. The parasitological techniques used were immunohistochemistry, histopathology, and culture with identification of the *Leishmania* species by isoenzymes from skin fragment samples. The animals that presented at least one of these positive tests were considered positive, and the animals with a negative result in all these tests were considered negative. The sample was randomly divided into two groups of equal sizes: one for creating the predictive model (Sample 1) and another for validating this model. Sample 1 was analyzed using univariate logistic regression, and the variables associated with the outcome (significance level < 20%) were analyzed using multivariate logistic regression, considering a significance level of 5%. The calibration of the clinical-laboratory model was evaluated using the Hosmer-Lemeshow statistics. We developed a scoring system by assigning

a score to each variable calculated by the ratio between the regression coefficient associated with each variable and the lowest regression coefficient among these. Sensitivities, specificities, and positive predictive values (PPV) and negative predictive values (NPV) and respective 95% confidence intervals (CI95%) were estimated for the different cutoff points of the clinical-laboratory score and the DPP® CVL rapid test. We used SPSS and Stata statistical software for the analyses. Only splenomegaly was not associated with the outcome in the univariate analysis (p-value: 0.76). The final model obtained through multivariate logistic regression was composed of four variables: weight loss (OR: 13.08; score: 3); furfuraceous scaling (OR: 2.47; score: 1); onychogryphosis (OR: 7.63; score: 2) and DPP-LVC (OR: 54.58; score: 4). The clinical-laboratory model showed good discriminatory power and demonstrated a good calibration. In the validation sample, the sensitivity and specificity of the model with only DPP-LVC was 90.00% (CI95%: 78.64-95.65) and 68.75% (CI95%: 65.15-72.14), respectively. The specificity of the clinical-laboratory model (92.05%; CI95%: 89.67-93.92) was higher than that found only for the DPP-LVC rapid test; however, there was a significant drop in sensitivity values (54.35%; CI95%: 40.18-67.85) when compared to the performance of the model containing only the DPP® CVL rapid test. The clinical variables that composed the final model are frequently associated with CVL in other studies, suggesting the potential applicability of the proposed model in veterinary practice. We concluded that adding clinical variables to the model with the DPP® CVL rapid test did not contribute substantially to increasing the predictive power of CVL.

Keywords LEISHMANIASIS, VISCERAL; DOGS; BRAZIL; SIGNS AND SYMPTOMS; DIAGNOSTIC TECHNIQUES; PROCEDURES

P1-007: NEOLEISH®, A SAFE AND EFFICACIOUS DNA VACCINE AGAINST CANINE LEISHMANIASIS

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Neoleish® is a DNA vaccine based on the non-replicative antibiotic resistance gene-free pPAL plasmid containing the *Leishmania infantum* activated protein kinase C receptor analog (LACK) gene. The absence of antibiotic resistance genes, aside from being a requirement of the European Medicines Agency (EMA), makes this vaccine suitable for storage and worldwide distribution without freezing. Three efficacy studies, including immunogenicity and protection, were conducted with a total of 108 beagle dogs in compliance with animal welfare legislation. The vaccine is administered following a prime-boost homologous regimen via the intranasal route with an interval of 15 days. The post-immunization immune response was assessed. The challenge was carried out with 10^8 metacyclic promastigotes of *Leishmania infantum* via IV 15 days after the boost, and samples were obtained on 0 and 53 days post-vaccination and 21, 120, 180, 240, and 300 days post-infection. The clinical profile was evaluated and the parasite load in bone marrow was determined by qPCR. Total IgG, IgG1, and IgG2a levels were quantified by ELISA. T cell response evaluation against LACK and the crude *Leishmania* antigen (CLA) was carried out by the

lymphoblastic transformation test (LTT) in PBMC throughout the experiment and in target organs (spleen, liver, and popliteal lymph node) at the endpoint. IFN-γ and IL-10 levels in LTT supernatants were quantified by ELISA. Statistical inference was performed using the Mann-Whitney U test ($\alpha = 0.05$). Pre-clinical development revealed that the overall efficacy of **Neoleish®** in beagle dogs is ~60%. The clinical score was significantly lower in vaccinated dogs compared to control dogs throughout the experiments. The parasite burden in bone marrow decreased ≥ 100-fold in ~60% of vaccinated dogs, of which two-thirds were negative throughout the experiments. Endpoint parasite burden reduction in spleen, liver, and lymph node was similar. Post-immunization pre-challenge circulating total IgG and IgG2a levels significantly increased. No significant differences between vaccinated and control dogs were found in total IgG and IgG subtype levels after the challenge. Significant post-immunization pre-challenge and post-challenge T $CD4^+$ cell activation in PBMC against LACK was detected in ~80% of vaccinated dogs. Similar post-challenge activation levels were observed against CLA. Endpoint T $CD4^+$ cell activation levels in target organs against CLA and LACK were significant in ~70% of vaccinated dogs. The IFN-γ concentrations in LTT supernatants from PBMC and target organs of vaccinated dogs were significantly increased and ranged between 20 and 50 pg/mL. On the contrary, IL-10 concentrations significantly decreased in the vaccinated groups compared to the control groups. The **Neoleish®** double-blind field trial was conducted in three geographical areas in Spain with the cGMP-produced vaccine and confirmed the pre-clinical development results. **Neoleish®** is a safe vaccine that confers ~60% of protection in terms of clinical sign and parasite burden reduction, activating a Th1 response.

Keywords DNA VACCINE; IMMUNE RESPONSE; *Leishmania infantum*; LACK GENE

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P1-008: CANINE VISCERAL LEISHMANIASIS: CLINICAL FEATURES AND DIAGNOSTIC EVALUATION IN NORTHERN URUGUAY

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Canine visceral leishmaniasis (CVL) is a parasitic and zoonotic disease caused by *Leishmania infantum*, being the dog the main reservoir in rural and urban areas. CVL is currently expanding over the American continent and since February 2015 the southernmost location where the disease was observed has being Arenitas Blancas (Salto, Uruguay). The aim of this research was to evaluate different diagnostic approaches in the areas of our country where CVL has recently arrived. This research was performed in the north of Uruguay at the cities of Salto and Bella Unión. Up to 205 dogs older than 6 months were selected during the active surveillance for CVL performed by the Ministry of Public Health, from July to December 2019. Age, race, sex, clinical symptoms, sleeping site and city were registered for each individual, allowing to group dogs according to different characteristics. Serum samples were obtained and evaluated using 4 different serologic techniques: rK39-ICT InBios, DPP Bio-Manguinhos (BM), ELISA Bio-Manguinhos and Elisa in house. PCR was also performed from lymph node aspiration samples by amplification of the *its-1* gene. All procedures used were approved by the Honorary Animal Experimentation Commission of the Facultad de Veterinaria (Nº 915-19). From the total of 205 dogs, 44,9% were positive to at least one of the described techniques. The positivity of each technique was: 32,7% for rK39, 41,5% for DPP, 30,2% for ELISA Bio-Manguinhos, 35,1% for the ELISA in house and 22,8% for PCR. When we evaluated the positivity of the techniques according to clinical

symptoms, the DPP was the best detecting asymptomatic dogs (28,2%), followed by ELISA BM (22,6%), ELISA *in house* (22,2%) and rK39 (20,9%). Regarding to the study population characteristics, 70,7% of the positive dogs were symptomatic (p=0,001), 48,2% used to sleep outdoors (p=0,04), 53% were males (p=0,04), and the proportion of positive animals was higher at Salto (77%, p<0.0001). There were no significant differences according to race or age. The most frequent clinical symptoms were lymphadenopathy (67%), weight loss (46%), dry seborrhea (44%), alopecia (42%) and onychogryphosis (37%) but only adeno and hepatomegalia are exclusive symptoms of positive population when compared to clinical signs of negative population. This work reinforces the good sensitivity showed by DPP when compared to rk39 regarding to detection of asymptomatic dogs suffering of CVL. Our results show that the cities studied have higher positivity rates than other cities of the continent where CVL has been established for longer time. In conclusion, Salto and Bella Unión show specific frequencies of clinical symptoms when compared to other cities which reinforces the need of further clinical characterization, males that sleep outdoors are the most vulnerable population regarding CVL and the combination of the different techniques is needed in order to fully detect CVL positive individuals.

Keywords: *Leishmania infantum;* DOGS; DIAGNOSIS

P1-009: SEROPREVALENCE OF CANINE LEISHMANIASIS IN PARAGUAY, 2018-2021

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Visceral leishmaniasis is a chronic parasitic disease caused by *Leishmania infantum*, transmitted by infected sandflies that affects humans and its urban reservoirs dogs. The purpose of this work was to determine the seroprevalence of canine leishmaniosis by immunochromatographic rK39 test in serum samples obtained for routine exam requested by dog`s owners and veterinarians, active surveillance in areas of silence transmissions and control of human cases notified for the National Service of Eradication of Vector-borne diseases (SENEPA) to the National Program of Zoonoses Control and Rabies National Center (PNCZyCAN) since 2018 to 2021. A total of 14.698 blood samples were analyzed by immunochromatographic rK39 (Kalazar Detect Rapid Test, canine. Inbios®, Seattle, USA), in the laboratory of Leishmaniasis of the PNCZyCAN, from all the 17 departments of the country. The 7.571 canine blood samples proceeding for routine exam from Asunción (capital city), Alto Paraguay, Alto Paraná, Amambay, Boquerón, Caaguazú, Caazapá, Canindeyú, Central, Concepción, Guairá, Itapúa, Cordillera, Misiones, Ñeembucú, Paraguarí, Presidente Hayes y San Pedro departments, showed 2.258 positive serum samples with a prevalence of (29.8%) of canine leishmaniasis. The active surveillance showed that 213 out of 3.272 samples had a positive result with a prevalence of (6.5%) of canine leishmaniasis. From the focus of human visceral leishmaniasis was observed that 242 out of 3.825 canine samples were positive with a prevalence of (6.3%). A global seroprevalence of (18.4%) of canine

leishmaniasis was observed. Euthanasia procedures were carried out in 1940 positive dogs (71.5%) from routine exam, focus of human visceral leishmaniasis and active surveillance in areas of silence transmission of visceral leishmaniasis. The high prevalence of canine visceral leishmaniasis shows the compelling need to continue a strict epidemiological surveillance, sanitary education and community participation by the Ministry of Public Health and Social Welfare in the control of this disease in Paraguay.

Keywords CANINE LEISHMANIASIS; SEROLOGY; rK39; EPIDEMIOLOGY; PARAGUAY

P1-012: PRESENCE OF CANINE VISCERAL LEISHMANIASIS IN A VILLAGE IN THE MUNICIPALITY OF GIRÓN, SANTANDER, COLOMBIA

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Canine visceral leishmaniasis in endemic areas of Colombia constitutes a risk factor for public health, taking into account the zoonotic nature of the disease; the presence of the epidemiological triad requires the immediate intervention of the territorial entity. In this sense, a focus study was carried out in the month of May 2022, with the objective of identifying the presence of vectors, reservoirs and environmental conditions for the transmission of visceral leishmaniasis (VL), in the village of Palo Gordo, a rural area of the municipality of Girón in the department of Santander, where a case of human VL was recorded in 2019. Joint field trips were carried out between the Secretary of Health of Santander (Santander Public Health Laboratory and ETV Program) and the Secretary of Health of Girón (ETV Program, zoonoses and epidemiological surveillance), for two weeks. Surveys of leishmaniasis risk factors were applied and blood samples were taken in humans (n=94) and canines (n=91) to set up the rK39 immunochromatographic test or Rapid Detection Tests (RDT) for Visceral Leishmaniasis and Indirect Immunofluorescence (IFI). Entomological surveillance was carried out, in order to determine the presence/absence of the vector in the study area, CDC traps were installed in intra-, peri- and extra-domicile on May 24, 25 and 26 between 18:00 and 06:00 the sampling was carried out with a coverage of 10 dwellings. RDT performed on humans were negative; of the 91 canines sampled, 14 were positive, these results were confirmed by IFI at the National Institute of Health. The entomological study confirmed the presence of the vector Lutzomia Longipalpis in 9 of the 10 houses sampled. Bearing in mind that the dog is the most important domestic reservoir within the transmission cycle due to its close contact with man and the presence of the vector in the area, alarms have been raised in the department's health authorities.

Keywords LEISHAMANIASIS; FOCUS; ZOONOTIC; VECTOR; RESERVOIR

P2-001: CLINICAL MANAGEMENT OF CANINE VISCERAL LEISHMANIASIS BY VETERINARIANS IN PARAGUAY

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Leishmaniasis is a zoonotic disease caused by several species of protozoa of the genus *Leishmania*, and transmitted to people and animals by the bite of sandflies infested with the parasite *Leishmania infantum* causes a severe disease, being the domestic dog considered as the main reservoir. The wide spectrum of clinical signs observed in dogs, as well as the correct and precise application of the various diagnostic methods, treatments and prevention and control measures, used by veterinarians, are necessary to perform a correct clinical management and reduce the incidence of this endemic pathology in Paraguay. The general objective of the research work was to evaluate the clinical management (clinical signs observed, as well as the methods of diagnosis, treatment, prevention and control) of canine visceral leishmaniasis applied by veterinarians of small animals from the metropolitan area of the city of Asunción. A questionnaire was carried out to a total of 200 veterinary professionals, with open and closed questions on the clinical management of canine leishmaniasis, with the city of Asunción being the most participatory with 92 professionals, 32 in San Lorenzo, 25 in Fernando de la Mora, 17 in Luque, 15 in Lambaré, 5 in Mariano Roque Alonso, 4 in Ñemby, 3 in San Antonio and Limpio and 2 in Capiatá and Villa Elisa, respectively. The results obtained showed that the most frequently observed clinical signs were: nodal hypertrophy (92%), weight loss (86%), nasal hyperkeratosis (83%), renal conditions (80%), eye lesions (75%), and alopecia (63.5%), mainly. The laboratory diagnosis of preference used by veterinary professionals is the immunoenzymatic ELISA test (96%)

followed by aspiration and microscopic examination of bone marrow (89%). Regarding the most suggested treatment, veterinarians use the single or combined administration of allopurinol (82%), and pentavalent antimonials (74.5%), domperidone (75%) and miltefosine in a (61%). For the control of animals seropositive to canine visceral leishmaniasis, euthanasia (70.5%) is recommended and; in terms of prevention and/or prophylaxis methods: insectids/repellents in the form of collars or spot on pipettes are most widely used (90%), annual serological analysis (81.5%) and vaccines against canine leishmaniasis in a (75.5%). It can be said that there is a good knowledge and correct management of canine visceral leishmaniasis by veterinarians of small animals in the metropolitan area of Asunción. Efforts should continue to implement preventive measures to reduce the incidence of this disease. Veterinarians of small animals should continue to be trained and updated in the clinical management of canine visceral leishmaniasis and educate dog owners on the correct application of preventive measures and control of this highly endemic disease in Paraguay.

Keywords CANINE LEISHMANIASIS; MANAGEMENT; PREVENTION; TREATMENT; PARAGUAY

P2-002: IMMUNOMODULATION OF CANINE MONOCYTE-DERIVED DENDRITIC CELLS BY *L.eishmania infantum* AND *L. amazonensis*

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Leishmaniosis a global zoonotic disease endemic in more than 70 countries. The dog can be infected by several species of *Leishmania*, including *L. amazonensis* although *L. infantum* is the most common etiologic agent of CanL found in Latin America, the Middle East, the Mediterranean basin, and Asia. Dendritic cells (DC) are crucial for the activation of the adaptive immune response. DC maturation depends on pathogen antigens recognition through pattern recognition receptors (PRR), such as Toll-like sensors, which can trigger the mechanism of action of nuclear transcription factor-kB (NF-κB) and translocation to the nucleus, which promotes transcription and synthesis of pro-inflammatory cytokines. Once activated, moDC can express chemokine CXCL16 in the cell membrane. In response to pro-inflammatory stimuli, the cell membrane binding is cleaved by the action of ADAM10 metalloprotease, and CXCL16 is released in its soluble form into the extracellular space. This chemokine interacts with the BONZO receptor (or CXCR16), promoting the adhesion and migration of T lymphocytes and natural killer cells. Thus, the present study aimed to explore the ability of dog monocyte-derived DC (moCD) to initiate the immune response after *Leishmania* recognition and its ability to induce leukocyte migration. moDC were differentiated in vitro and exposed to *L.*

infantum and *L. amazonensis* promastigotes for 24h. In parallel, soluble antigen and extracellular vesicles (EVs) shed by both species of *Leishmania* were used to stimulate moDC. The morphology of moDCs was examined by scanning electron microscopy, the gene expression of cytokines and cell sensors were analyzed by qPCR, and the constitution and translocation of NF-κB to the nucleus and the production of CXCL16 were evaluated by immunoassays. After exposure to *L. infantum* and *L. amazonensis* promastigotes, moDC differentiated multiple dendrites and exhibited high TLR2 gene expression. Nevertheless, TLR4 only was triggered by *L. infantum* and TLR9 by *L. amazonensis*. After sensing of *Leishmania* parasites, high activity of NF-kB was shown, although without any detectable effect on cytokine generation. Nevertheless, soluble chemokine CXCL16 increased when compared with resting moDCs. After stimulation with *L. infantum* and *L. amazonensis* antigens, moDC augmented TLR2 gene expression. *L. infantum* antigen promoted the generation of IL-12p40 and *L. amazonensis* antigen enhanced IL-10 and IL-18 gene expression. EVs purified from both *Leishmania* species promoted the release of CXCL16. In both antigen and EVs stimulation, moDCs evidenced NF-kB enrichment. *Leishmania* species causing visceral and cutaneous leishmaniosis were recognized by canine moDC, leading to the activation of different downstream pathways, culminating with the translocation of NF-kB, which controls DNA transcription and cytokine production. In contrast with parasite antigen, parasite EVs do not seem to be sensed by PRR. Nevertheless, parasite EVs modulate moCD by activating NF-KB. In addition, activated canine moDC can drive T lymphocytes migration. These findings can raise new possibilities for personalized medicine intervention regarding visceral and cutaneous leishmaniosis.

Keywords CANINE LEISHMANIOSIS; MONOCYTE-DERIVED DENDRITIC CELLS; *Leishmania* EXTRACELLULAR VESICLES; INNATE IMMUNITY

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P2-003: ACCORDANCE DEGREE BETWEEN FOUR SEROLOGICAL TESTS FOR *Leishmania infantum* ANTIBODIES DETECTION IN NATURALLY INFECTED DOGS IN MINAS GERAIS, BRAZIL

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Leishmaniasis precise diagnosis is the first step to correctly staging the disease in dogs (LCan). Complete identification of *Leishmania* spp. in host biological tissues is possible to be accessed by parasitological approaches mainly molecular ones. However serological tests are very useful in monitoring antibodies levels and helpful to estimate humoral response of vertebrate host besides being costless and easy to perform. Rapid tests (RTs) are screening methodologies also widely performed in veterinary routine as the initial step in detection of *Leishmania*-positive patients. After that, more specific and sensible serological tests as ELISA and RIFI are performed in order to corroborate RTs result. In this work was evaluated the accordance between VETLISA LEISHMANIOSE IgG (Bioclin Vet) a commercial kit for anti-*Leishmania* IgG detection, RT ELISA SNAP Leish Idexx® (Maine, USA), and ELISA and RIFI in house assays performed in Laboratório de Leishmanioses (ICB-UFMG). In this sense, 275 serum samples from dogs attended in Santo Agostinho Hospital Veterinário (Belo Horizonte, Brazil) were enrolled in the comparative study. The accordance between VETLISA and RT ELISA SNAP Leish Idexx® was 85.1% (234/275), between VETLISA and in ELISA (UFMG) 81.8% (225/275), and 86.2% (237/275) between VETLISA and RIFI (UFMG). RT ELISA SNAP Leish Idexx® agreed with in house ELISA (UFMG) and RIFI (UFMG) in 84.7% (233/275) and 85.5% (235/275) of the samples, respectively; while the agreement between both in house ELISA (UFMG) and RIFI (UFMG) tests, the agreement was about 89.1% (245/275). These results demonstrated that the agreement among all tests analyzed was quite expressive (above 81.0% for

all of them); RT ELISA SNAP Leish Idexx® could be employed as serological screening test while RIFI (UFMG) plus in house ELISA (UFMG) or VETLISA as confirmatory tests, increasing sensibility of anti-*Leishmania* antibodies detection, mostly when combined with parasitological tests. Taken together all the results raised evidence that all of them can be employed in veterinary clinic routine in diagnostic of LCan and in monitoring dogs being treated for leishmaniosis. The development of more accurate diagnostic tools that optimizes and accelerates *Leishmania* spp. detection and LCan treatment is extremely relevant, especially in the context of neglected tropical diseases, a challenge and a concerning worldwide.

Keywords DOG; LEISHMANIASIS; SEROLOGICAL DIAGNOSTIC, TEST AGREEMENT

P2-004: OCCURRENCE OF COINFECTION WITH TICK-BORNE PARASITES IN DOGS NATURALLY INFECTED WITH VISCERAL LEISHMANIOSES IN MINAS GERAIS STATE, BRAZIL

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Canine leishmaniasis (LCan) and the hemoparasitosis, a complex of tick-borne diseases that can be caused by microorganisms such as *Anaplasma, Babesia* and *Ehrlichia* genus, are of great relevance and concerned worldwide in the context of dog health. Together they consist in the most prevalent infective diseases in veterinary clinical routine, and accurate diagnosis it is a great challenge in patient management. Besides, it is well known that precise diagnosis is based in the complementary of serological and parasitological techniques, and for the latter, molecular assays are the most sensitive ones. LCan and the hemoparasitoses can cause hemodynamic disorders through interference of sanguineous compounds production or by leading it destruction. Independently of the pathological process involved, as a consequence can be observed unspecific clinical signs that interfere in the correct etiological agent detection. More frequent than is known, coinfections among hemoparasites and LCan may occur and be underestimate. In this sense, was investigated the frequency of positivity in naturally infected dogs, attended in Santo Agostinho Hospital Veterinário (Minas Gerais, Brazil) for one or more hemoparasitoses through molecular diagnosis with PCRun® QUATTRO Tick-Borne (Biogal, Israel) and LCan in ELISA and RIFI in house assays developed by Laboratório de Leishmanioses (ICB-UFMG) and SNAP Leish Idexx® (Maine, USA). Twenty-six dogs, in which was detected gDNA from one or more hemoparasite (*A. platys*, *B. canis*, *B. gibsoni* or *E. canis*) in whole blood sample by PCRun reaction had it chart and serological tests for anti-*Leishmania* antibodies detection results recovered from the hospital database. From the total samples tested by

PCRun in 26.9% (7/26) was also detected anti-*Leishmania* IgG in at least one or more serological tests, and from that in 100.0% (7/7) was concomitantly detected *E. canis* through PCR. Furthermore, in one sample (14.3% - 1/7) was found *E. canis* and *A. platys* DNA in PCR, and sera reactivity in house RIFI, ELISA and in SNAP Leish Idexx® for *L. infantum*. These results showed that *E. canis* was the most prevalent hemoparasite detected in the analyzed samples and reinforce the idea that coinfection between LCan and hemoparasites is occurring in field conditions and may be underestimated. Coinfected individuals probably may have more severe clinical signs, weakness in immune system, and a poor prognosis. These study raises evidence about the importance to investigate comorbidities in LCan and in ehrlichiosis patients by employing more sensible and specific diagnostic tools.

Keywords DOG; LEISHMANIASIS; MOLECULAR DIGNOSIS, COINFECTION, EEHRLICHIOSIS

P2-005: SENSITIVITY OF CYTODIAGNOSTIC STUDIES AND RELEVANCE OF CLINICAL FINDINGS IN THE DIAGNOSIS OF CANINE VISCERAL LEISHMANIOSIS

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The city of Posadas, Misiones, Argentina, is an endemic area for Canine Visceral Leishmaniosis (CVL) caused by *Leishmania infantum* transmitted by *Lutzomyia longipalpis,* since 2006. In dogs, the disease affects almost every system or organ, which makes the clinical diagnosis particularly complex. Its timely diagnosis is very important for the individual patient but also epidemiologically since, in this way, through proper treatment, it is possible to reduce the circulation of the parasite in both the animal and human population. Within the 1637 dogs analysed between 2006 to 2018 in a veterinary clinic in the city, animals with varied presentations were found. The main clinical manifestations were: various skin conditions, onychogryphosis, blepharitis, uveitis, lymphadenomegaly, hepatosplenomegaly, renal failure. 1150 CVL positive patients were selected; whose samples were taken from different organs. 1070 dogs were sampled by fine needle aspiration (FNA) of superficial lymph nodes. Skin samples of suspected animals were 708 samples using scrapes and/or imprints of various lesions, as well as skin nodule FNA. There were obtained 238 samples of bone marrow, using FNA at costochondral junction and/or sternum. The smears were dyed with rapid panoptic staining and observed under the optical microscope with a 100X lens using immersion oil. Positive samples were given with the presence of amastigotes of *Leishmania spp.* in the smears. The negative, after observing 400 fields in representative samples. 85.88% (919 dogs) tested positive for superficial lymph node samples. Amastigotes were found in 267 skins, which means that 37.71% of

the samples were positive. Regarding bone marrow, the finding was 218 positive samples, 91.59% of all dogs examined. Lymph node performance was acceptable, being the most used site, given the experience in obtaining good quality samples with easy accessibility. Skin samples had low sensitivity, except in skin nodules or active ulcers, since in chronic condition, the observation of *Leishmania spp.* was occasional. Although the bone marrow was rarely used at first, due to its more complex sample taking process, it ended up being the location with more sensitivity. Today in our practice, it is the organ of choice since more skills has been acquired for its implementation. In addition, many animals now come to the consultation with fewer symptoms and do not always present with adenomegaly. Given the disparate sensitivity of parasitological tests, adequate pre-test clinical selection improves the diagnostic performance of laboratory studies. Ideally, two or more organs should be sampled for more reliable results.

Keywords DOGS; PARASITOLOGICAL DIAGNOSIS; NORTH-EASTERN ARGENTINA

P2-007: EPIDEMIOLOGICAL MONITORING OF *Leishmania* AMONG HUMANS, VECTORS AND CANINES: A SURVEILLANCE STUDY WITH MOLECULAR AND GEOGRAPHIC ANALYSIS IN CARTAGENA

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Leishmaniasis is a vector-borne disease caused by various species of the *Leishmania* taxonomic complex. In Colombia, leishmania infections are mostly endemic in rural areas, and yet, they remain neglected infectious diseases because they are under-studied, under-diagnosed, and under-reported. The urban transmission is driven by the distribution of vectors (such as *Lutzomyia* species), susceptibility of domestic reservoirs (such as dogs), and environmental and sociodemographic factors. However, despite numerous reports globally, epidemiological information associated to leishmaniasis in Colombian urban areas remains scarce and poorly understood. Here, we report the detection and characterization of *Leishmania* species in humans, domestic dogs and vectors, as well as the spatial distribution of cases in two urban, forested, and poverty-stricken areas of Cartagena de Indias. During October 2021, we conducted an epidemiological surveillance in "La Quinta" and "El Toril" neighborhoods using a convenience sample. Subjects were enrolled in collaboration with community leaders of both neighborhoods. Demographic surveys were applied, and a total of 118 and 52 blood specimens were collected from humans and their domestic dogs, respectively. Genomic DNA was extracted, and the internal transcribed space 1 (ITS1) of *Leishmania* species was PCR-targeted. PCR products were sequenced by Sanger method, and nucleotide

sequences were submitted to BLASTn and then analyzed using phylogenetic tools to confirm the molecular identification. Of the human samples, 4.2% (5/118) were positive for *Leishmania donovani* complex; 5.8% (4/69) in “La Quinta”, and 2.0% (1/49) in “El Toril”. All dog samples were negative for the ITS1 PCR assay. Three CDC light traps were placed during three consecutive days between 18:00 and 6:00 in peridomiciliary areas of the *Leishmania*-positive human case living in "El Toril". Due to security issues, the other neighborhood was not included in the entomological surveillance. The entomological survey resulted in the identification of six female *Lutzomyia* sand flies. All *Lutzomyia* sand flies were ITS1 PCR-negative. The spatial distribution of human cases was assessed using a georeferenced database that also included GPS coordinates of CDC light traps and flight ranges of *Lutzomyia* sand flies that were described elsewhere. Interestingly, most of the human cases were located within the flight range, and located near forested areas. Overall, despite the failure to detect *Leishmania* in vectors and domestic dogs, our results contribute important information that fills a void with regards to the epidemiology of Leishmaniasis in urban areas with a high risk of transmission, and highlight the need to expand the surveillance and identification of reservoir hosts.

Keywords *Leishmania*; *Leishmania donovani* COMPLEX; EPIDEMIOLOGY; CARTAGENA; COLOMBIA

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P2-008: EPIDEMIOLOGICAL SURVEY OF CANINE LEISHMANIASIS IN AN AREA OF SOUTHERN FRANCE

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Based on serological and molecular (PCR) studies, the prevalence of canine leishmaniasis and asymptomatic carriage of *Leishmania infantum* in endemic areas of the world can reach 80%. In southwestern Europe, nearly 2.5 million dogs are thought to be infested. In the south of France, one of the major focus is located in the Cevennes region, and seroprevalence in dogs varies from 5.51% to 17.9% according to previous studies, last of which was carried out in 2003. The objective of this work was 1) to evaluate the current endemicity of canine leishmaniasis in this area 2) to assess the use of the prophylactic measures implemented (anti-vectorial protection and vaccination 3) to compare different serological tests. Material and Methods. Population and samples: Between December 2021 and January 2022, 170 dogs were sampled including blood (for serology and PCR) and oral swab (for PCR). Five kits were evaluated for the serological diagnosis, 3 ELISA methods (ID Screen® Leishmaniasis Indirect Innovative Diagnosis; *Leishmania infantum* Bordier Affinity products; VetLine Leishmania Novatec Immundiagnostica GmbH) and 2 immunochromatography tests (Speed Leish K™ Virbac; FASTest® Leish Megacor). Ethical approval N° 2021070114534565. Results. The 170 dogs (24 breeds + crossbreeds) belonged to 17 owners and lived in 12 localities (over 1200 km^2). 88.2% were hunting dogs, 5.9% were companion and breeding dogs. The sex ratio M/F was 0.92. 17.2% of the dogs studied were receiving effective external antiparasitic protection against sandflies during the transmission season. 83.5% had never been vaccinated. Only 0.9% were vaccinated and received vector prophylaxis. 3.6% had clinical signs consistent with clinical

leishmaniasis and 9 dogs (5.3%) had been previously diagnosed with canine leishmaniasis. According to the technique, the seroprevalence for all dogs (n=170) and for non-vaccinated dogs (n=142) varied from 15,8% to 30,6%, and from 14.7 to 23% respectively. A revision of the positivity thresholds for ELISA methods is proposed. PCR analysis are ongoing. Conclusion. This study shows that the use of efficient anti-sandfly protection and vaccination is low, while the owners of the dogs are aware of the protection methods. Regarding serological test, in the absence of a gold standard, it is difficult to determine the best test to diagnose disease or asymptomatic carriage. Interpretation of positive serological tests in vaccinated dogs is a real question. Comparison with clinical and PCR data and long-term follow-up of these dogs should allow a better estimation of the accuracy of the serological tests.

Keywords CANINE LEISHMANIASIS; SOUTH FRANCE; EPIDEMIOLOGY; SEROLOGY; PCR

Financing Santé Publique France

P2-009: RISK FACTORS ASSOCIATED WITH THE OCCURRENCE OF CANINE VISCERAL LEISHMANIASIS IN METROPOLITAN AREAS OF THE CITIES OF ASUNCIÓN AND SAN LORENZO, PARAGUAY

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Canine visceral leishmaniasis is an endemic disease of great public health concern, mainly in Latin America and Paraguay. A case-control study was carried out, with the aim of associating risk factors with the occurrence of visceral leishmaniasis, for which 196 dogs were sampled, that attended the Veterinary Hospital and a Veterinary Center in the cities of San Lorenzo and Asunción. Of the canines sampled, 98 were cases and 98 controls (Polymerase Chain Reaction positive and negative). Subsequently, a questionnaire provided to the owners was used to obtain data related to the exposure or not of the canines to the risk factors. For the statistical analysis, the program for epidemiological analysis of tabulated data EPIDAT Version 3.1 was used, obtaining the following results: taking into account the demographic characteristics of the canines: breed (mixed or purebred) (OR=0.92; p= 0.8); age (adults or puppies) (OR=0.8; p=0.7); sex (male or female) (OR=0.96; p=1.0); and function of the dog (guard or companion) (OR=0.75; p=0.5); Regarding the factors related to animal management: lack of vaccination (OR=24.8; p=0.000; staying away from home (OR= 4.18; p=0.0037; staying away from home at night (OR = 2.3; p=0.0082, lack of use of repellents (OR= 1.13; p=0.7 and non-fumigation of the home (OR= 0.78;

p=0.47 and according to the factors dependent on the environment in which the canines live: access to a vacant yard (OR=1.94; p=0.05); ownership of a yard or garden (OR=0.60; p=0.56); proximity to a water source (OR=0.56; p=0.22); ownership of a chicken coop or pigsty (OR=0.32; p=0.61); proximity to garbage dumps (OR=2.03; p=0 ,18), being considered as risk factors for the occurrence of canine visceral leishmaniasis: lack of vaccination, stay away from home, stay away from home at night and access to vacant patio. These results contribute to the knowledge of these factors in the area, aiming at the implementation of prevention and control measures.

Keywords CANINE LEISHMANIASIS; RISK FACTORS; PARAGUAY

P2-009.1: DOGS UNDER TREATMENT FOR CANINE LEISHMANIASIS ARE MORE PREDISPOSE TO XANTHINE UROLITHS FORMATION: A RETROSPECTIVE STUDY FROM MINAS GERAIS, BRAZIL

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The use of allopurinol in canine leishmaniosis (LCan) treatment is frequent reported as a cause of urolithiasis. However, uroliths composition has not uniformly recorded in analyzes performed in different laboratories. In this sense, data from patients chart attended in Santo Agostinho Hospital Veterinário was accessed in order to evaluate the occurrence and composition nature of uroliths found in 33 dogs naturally infected by *Leishmania infantum* in the period from 2005 to 2021. This retrospective study shown that 66.7% (22/33) of the dogs were male and 33.3% (11/33) were female; and uroliths of the highest incidence was urate (39.4% - 13/33), followed by xanthine (21.2% - 7/33). Besides, calculi of other natures, one of struvite and the another one of mixed composition, were identified in two infected males, that were not been treated with allopurinol. Related to breed predisposition, the uroliths were more frequent detected in 9/33 mongrel dogs (27.3%) and in 5/33 Labrador Retrievers (15.2%). Taken all the results together was observed that males presented more frequently urate uroliths (9/22 – 40.9%) and xanthine (6/22 – 27.3%); and in females struvite (4/11 – 36.4%) especially in Schnauzers (3/4 – 75%). Moreover, identification of xanthine calculi varied according to the methodology employed in its analysis suggesting that the technique used in each laboratory can directly influence in xanthine uroliths identification. The physical-chemical methodology for uroliths analysis, which characterizes the total chemical composition of the stone and its

macroscopic characteristics, showed less precision when compared to the techniques of energy dispersive spectroscopy, optical crystallography or infrared spectroscopy. These ones provide the stone layers composition with greater precision increasing sensitivity in identify the presence of xanthine in the stones composition. In this way, this study suggested that the choice of methodology for stone identification direct influences in it correct categorization, enabling the clinician establishing the correct therapeutic management of patients undergoing continuous treatment with allopurinol.

Keywords DOG; LEISHMANIASIS; ALLOPURINOL; UROLITHS

P2-076: PRODUCTION OF AN ANIMATED VIDEO FOR SCIENTIFIC LITERACY, PREVENTION OF VISCERAL LEISHMANIASIS AND ADOPTION OF RESPONSIBLE ANIMAL GUARDIANSHIP

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The creation of pedagogical films aimed at Health Education aims to systematize and favor the construction of knowledge for a transformative educational practice. The videos can also bring together scientific and artistic languages and show the contribution of art in the process of Scientific Literacy. In this perspective, the objective was to produce an audiovisual work aimed at dog tutors in areas where animal abandonment and the movement of unrestricted dogs constitute relevant problems at the public health level. Thus, were contemplated the themes of: animal welfare; canine visceral leishmaniasis and responsible companion animal guardianship. To this end, a group of undergraduate and graduate students from different areas of health carried out a search for scientific articles dealing with the subject in different databases (MEDLINE, SciELO, PubMed and LILACS). After compiling the contents to be covered, the members listed 10 topics in a language accessible to the general public and built an animation with audiovisual effects. A video lasting 3 minutes and 23 seconds was prepared, which among other topics, addressed the relationship of responsible animal guard with canine visceral leishmaniasis. The material built had as main characteristics the graphic presentation of situations that promote the reflection of the problem; sound effect capable of sensitizing the spectator and practical recommendations to be adopted by the tutors of companion animals. The material made emphasizes that non-compliance with the principles of responsible animal care can have effects on public

health, including the greater probability of tutored animals without these behaviors, presenting greater chances of disseminating Visceral Leishmaniasis in endemic areas. The video is being applied in an intervention study carried out in an endemic area and has been positively evaluated by tutors and the community. Thus, our study integrated different fields, methods and knowledge, with the improvement of aspects still little explored about the adoption of responsible companion animal guardianship. The analyses, carried out in the field of language studies, contribute to the development of educational projects that provide the establishment of connections between science and the arts, helping to disseminate health education, environmental and humanitarian education.

Keywords ARTSCIENCE, ANIMAL WELFARE; HEALTH EDUCATION; VISCERAL LEISHMANIASIS

Financing The study was funded by National Council for Scientific and Technological Development (CNPq)

P3-001: MORTALITY RATE IN CANINE LEISHMANIOSIS: A 20-YEAR RETROSPECTIVE STUDY

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Canine leishmaniosis (CanL) caused by *Leishmania infantum* is a zoonotic vector-borne disease endemic in the Mediterranean Basin. Dogs are the main peridomestic reservoir for the infection and can also succumb to the disease. This retrospective study aimed to evaluate the mortality rate in dogs with CanL from 2000 to 2020. Data of dogs with CanL, that attended the consult of infectious and parasitic diseases of the Veterinary teaching Hospital at the Universidad Complutense de Madrid were analyzed. Information about the breed, preventive measures, habitat, age, sex, and LeishVet clinical stage[1] were extracted from the clinical records to determine its association with mortality in CanL. A total of 1194 clinical records from dogs with CanL were analyzed. Along the 20 years included in our study, 84 dogs died of leishmaniosis, resulting in an annual mortality rate of 0.35%. Our data show a reduction in mortality from 2000 to 2015 (7.63%) compared with 2016 to 2020 (4.41%) (Figure 1). We also found a significant association between the mortality rate and age, with geriatric animals showing a higher mortality rate (10.67%), when compared to adults (6.17%) and young dogs (5.86%) (p= 0.03882). Dogs in LeishVet stage IV presented the highest mortality rate (38.89%) in comparison to dogs in stage III (7.65%), II (3.66) and I (1.51%) with significant difference between groups (p<0,001). Dogs infected with *L. infantum* can show no clinical signs for months or even years or develop the disease, with different degrees of severity. The outcome depends on the ability of its immune system to control the infection[2]. A weaker immune system and the presence of comorbidities that can impair the immune response are probable causes for the higher mortality observed in older dogs. The leading cause of death in CanL continues to be chronic kidney disease. This data reinforces the

importance of an early diagnosis of glomerulonephritis in dogs with CanL to establish a prompt treatment following the IRIS guidelines. Our data show a decrease in mortality from 2015 to 2020 compared to the previous 15 years, probably due to improved veterinary care, awareness campaigns and stronger dog-owner bond. A more in-depth analysis should be made to confirm these hypotheses. The data obtained in this retrospective study is essential to understand the trends in CanL mortality over a long period of time in dogs living in endemic areas.

Keywords DOGS; *Leishmania infantum*; KIDNEY DISEASE

P3-002: EFFECT OF BREED AS AN INTRINSIC RISK FACTOR FOR SEVERE FORMS OF CANINE LEISHMANIOSIS: A RETROSPECTIVE STUDY OF 1.194 CASES

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The development of clinical leishmaniosis mainly results from the parasite down modulation of host protective immune response through multiple host-parasite interactions. However, these interactions can be affected by a wide range of factors, especially those involved in the immune activity of the host. Thus, we hypothesized that dog breed could be an important risk factor influencing susceptibility to canine leishmaniosis (CanL), as loss of genetic diversity in purebred dogs may decrease the diversity in immune system genes and compromise their functionality. The aim of this work was to investigate the effect of breed as a risk factor associated with: (i) the acquisition of the infection, and (ii) the development of severe forms of CanL. So, we carried out a retrospective study of the clinical data of 28,818 dogs attending the Complutense Veterinary Teaching Hospital, between 2000-2020. A total of 1,194 dogs were diagnosed with CanL at the Consultant of Infectious Diseases, based on compatible clinical signs, quantitative serology, cytology and/or PCR results for *Leishmania infantum*. A multivariable logistic regression model was built using R (cutoff p-value < 0.05). The odd ratios (OR) and their statistical significance (Fisher's Exact Test) were also calculated for those breeds with more than 25 individuals and at least 2 cases of CanL, using mongrel dogs as control group. In addition, we evaluated the potential association between breeds with high risk (OR > 1) to acquire the infection and their habitat (outdoor vs indoor), and the clinical stage they displayed, ranging from mild disease (stage I) to very severe disease (stage IV), based on LeishVet guidelines. These analyses resulted in the identification of 25 breeds at high risk of acquiring the infection, such as: Boxer, Alaskan Malamute, English Cocker Spaniel, Briard,

Pointer, Rottweiler, among others; but 5 of them (German Shorthaired Pointer, Spanish Mastiff, German Shepherd, Belgian Shepherd and Giant Schnauzer) were significantly associated with outdoor habitats, suggesting that their increased risk could be the result of a higher exposure to the vector. In contrast, 12 breeds were at low risk of acquiring the infection (e.g. Yorkshire Terrier, West Highland White Terrier, French Bulldog, Poodle), and no significant associations were found with their habitat. Interestingly, we also observed a significant decrease of these less susceptible breeds during the last seven years, which may have an impact on the co-adaptation process of *L. infantum*. Finally, we found 16 breeds significantly associated with the development of severe disease (stages III-IV), including: Boxer, Rottweiler, German Shepherd, Basset Hound, Catalan Sheepdog, Garafian Shepherd, among others. Those of these breeds that were not linked with an outdoor habitat are more likely to display susceptible genotypes for severe forms of canine leishmaniosis. We can thus conclude that breed may play a relevant role in the epidemiology of CanL and further research is needed to fully understand the influence of breed-associated genetic variants on the pathophysiology of the disease. Identification of these variants could contribute to control *Leishmania* infections.

Keywords *Leishmania infantum*; BREED PREDISPOSITION; SUSCEPTIBILITY; GENETICS; EPIDEMIOLOGY

P3-003: OCCURRENCE OF UROLITHS IN DOGS INFECTED WITH *Leishmania infantum*, IN THE PERIOD FROM 2005-2021

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The use of allopurinol in canine leishmaniosis (LCan) treatment is frequent reported as a cause of urolithiasis. However, uroliths composition has not uniformly recorded in analyzes performed in different laboratories. In this sense, data from patients chart attended in Santo Agostinho Hospital Veterinário was accessed in order to evaluate the occurrence and composition nature of uroliths found in 33 dogs naturally infected by *Leishmania infantum* in the period from 2005 to 2021. This retrospective study shown that 66.7% (22/33) of the dogs were male and 33.3% (11/33) were female; and uroliths of the highest incidence was urate (39.4% - 13/33), followed by xanthine (21.2% - 7/33). Besides, calculi of other natures, one of struvite and the another one of mixed composition, were identified in two infected males, that were not been treated with allopurinol. Related to breed predisposition, the uroliths were more frequent detected in 9/33 mongrel dogs (27.3%) and in 5/33 Labrador Retrievers (15.2%). Taken all the results together was observed that males presented more frequently urate uroliths (9/22 – 40.9%) and xanthine (6/22 – 27.3%); and in females struvite (4/11 – 36.4%) especially in Schnauzers (3/4 – 75%). Moreover, identification of xanthine calculi varied according to the methodology employed in its analysis suggesting that the technique used in each laboratory can directly influence in xanthine uroliths identification. The physical-chemical methodology for uroliths analysis, which characterizes the total chemical composition of the stone and its

macroscopic characteristics, showed less precision when compared to the techniques of energy dispersive spectroscopy, optical crystallography or infrared spectroscopy. These ones provide the stone layers composition with greater precision increasing sensitivity in identify the presence of xanthine in the stones composition. In this way, this study suggested that the choice of methodology for stone identification direct influences in it correct categorization, enabling the clinician establishing the correct therapeutic management of patients undergoing continuous treatment with allopurinol.

Keywords DOG; LEISHMANIASIS; ALLOPURINOL; UROLITHS

P3-004:CONGENITAL CANINE LEISHMANIOSIS IN ASUNCION, PARAGUAY.

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Leishmaniasis is an endemic disease registered in several countries of the world. It affects different species of domestic and wild animals, being the canines its main reservoir. The etiological agent is a protozoan belonging to the order Kinetoplastida, family Trypanosomatidae, genus *Leishmania*, which is capable of developing different types of clinical presentations: cutaneous, mucocutaneous and visceral. The study presents the case of a 5-year-old adult canine, Labrador breed, diagnosed with Leishmaniasis without treatment. She had 8 cubs, of which 4 died at 30 days of age. The owner manifested 2 episodes of serous vomiting, no relevant alterations were found on clinical inspection. Supportive fluid therapy was carried out and hours after hospitalization the patient died. A necropsy of the pup was performed. In the anatomopathological study, hepatomegaly, splenomegaly, increased mesenteric lymph nodes, nephromegaly were observed macroscopically; congestive lungs, left ventricular hypertrophy. Histopathologically, lymphoplasmacytic infiltrate with hyperplasia of the follicles with the presence of structures compatible with *Leishmania* spp amastigotes was evidenced in the spleen and lymphoid nodules. Multiple foci of lymphoplasmacytic-type inflammatory infiltrate were found in the liver, with hydropic degeneration and centrilobular necrosis areas. The kidneys presented interstitial lymphocytic inflammatory infiltrate. Leishmaniasis is classically described as a vector-borne disease, alternative methods of transmission, including horizontal (via direct blood-to-blood or sexual contact) and vertical (transplacental or transmammary) transmission, are likely to have an important role during transmission. Visceral leishmaniasis is the one that can be transmitted congenitally and, according to the World Health Organization (WHO), is one of the seven most important tropical diseases and represents a major public health problem

due to the variability of its presentation. clinical and potentially fatal outcomes. Previous studies have shown the congenital transmission of visceral leishmaniasis in humans and dogs, manifesting their status as carriers of the pathology with a wide distribution of the causal agent in various organs, reflecting transplacental transmission as an alternative route. The histopathological findings of the study support the parasitological diagnosis of visceral leishmaniasis and confirm the infectious nature of the acute clinical picture with fatal outcome. The data presented raise new challenges and considerations for the control of leishmaniasis transmission, considering that disease prevention methods that target only the vector may not be sufficient to control the spread of the disease.

Keywords CONGENITAL; CANINE; LEISHMANIASIS; PARAGUAY

P3-005: DEVELOPMENT OF QUANTITATIVE PCR (qPCR) AND DROPLET DIGITAL PCR (DDPCR) FOR THE QUANTIFICATION OF *Leishmania infantum* IN DOGS

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Visceral Leishmaniasis (VL) is a neglected disease caused by *Leishmania infantum* and is widespread in several parts of the world. Dogs are reservoirs; they may show severe clinical signs, and assist in the spread of the parasite, that are transmitted by the bite of vector insects (sandflies) such as *Phlebotomus* and *Lutzomyia*. Molecular data demonstrate the relationship of dogs in the leishmaniasis transmission chain. Results of studies carried out even in restricted locations have revealed a genetic diversity among protozoa that varies between symptomatic or asymptomatic animals. Cycles of transmission occur independently in humans, dogs and wildlife. Laboratory tests are constantly used to diagnose or study disease progression in dogs. Both conventional PCR and quantitative PCR (qPCR) can be used. For research purposes, qPCR can still be used to quantify the parasitic load in the animal. Droplet Digital PCR (ddPCR) is another useful tool for the diagnosis and research of *L. infantum*. Unlike other methodologies, the sample is partitioned so that amplification occurs separately within a tube or chip. The genetic material is amplified from single molecules and a Poisson distribution is then used to calculate the number of copies. Literature is scarce in reports detailing the qPCR and ddPCR methods in the diagnosis of *L. infantum* in samples taken from dogs displaying clinical sings. Current study was aimed at developing and validating qPCR and ddCR for detection and quantification of *L. infantum* in

dogs. The ddPCR was performed according to the manufacturer's recommendation and in accordance with that described by previous publications. A total of 39 clinical spleen samples from dogs were collected after necropsy of animals confirmed a positive diagnosis. The use of the samples was previously approved by the ethics committee. Lymphadenopathy, hepatomegaly, and splenomegaly were analyzed and rated on a scale as normal, mild, or advanced to generate a score (Score I). A second extended score (Score II) was generated in a similar way, not only using the previous parameters but also these as presence or absence: opaque coat, alopecia, flaking, hyperkeratosis, weight loss, onychogryphosis, eye discharge, corneal opacity, blindness, paresis legs, pale mucosa, ulcers. There was 100% agreement between the results of the tested samples. The number of copies detected by ddPCR correlated with the Cq found in qPCR. There was no correlation between copy number and rate or the presence of clinical signs (symptomatic and asymptomatic). The tools developed in this work are important for the research of *L. infantum* in humans or dogs. Studies on the course of the disease continue to be important to elucidate the mechanisms of interaction between host and parasite, as well as to evaluate the functioning of treatment and vaccines. Digital PCR is a new tool that can generate even more in-depth information in the debate on parasitic loads and the pathogenesis of leishmaniasis.

Keywords LEISHMANIASIS; DIAGNOSIS; PCR; HISTOPATHOLOGY

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P3-008: A POSSIBLE INDIRECT MARKER OF *Leishmania* spp. EXPOSURE IN DOGS

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Phlebotomine sand flies are vectors of *Leishmania* spp. parasites (Kinetoplastida: Trypanosomatidae), which are transmitted to a susceptible host during the blood meal of infected female phlebotomine sand flies. At the same time, immunogenic salivary proteins are deposited in the host, inducing an immune response with antibody production. The detection of antibodies produced against salivary antigens in the host allows estimating the host's exposure to phlebotomine sand fly bites in areas where leishmaniasis is endemic. *Lutzomyia migonei* is a proven vector of *Leishmania* (*Viannia*) *braziliensis* and permissive vector of *Leishmania infantum*. Studies aimed at detecting anti-saliva antibodies in dogs have been carried with some phlebotomine sand fly species, but not with *Lu. migonei*. The objective of this study was to detect antibodies to *Lu. migonei* salivary antigen in sera of dogs from an area where visceral and cutaneous leishmaniasis are endemic and *Lu. migonei* is present. Serum samples from 24 dogs, which were positive to anti-*Leishmania* antibodies using an immunochromatographic rapid test, were tested by an enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies to *Lu. migonei* salivary antigens. Serum samples were tested at a 1:50 dilution and the conjugate was used at a 1:8000 dilution. All *Leishmania*-positive samples were also positive to anti-saliva antibodies, resulting in a positivity of 100%, confirming the exposure of the dogs to *Lu. migonei*. Our ELISA was able to detect antibodies to *Lu. migonei* saliva in dogs, thus representing a new tool to assess the exposure of dogs to this vector and, indirectly, the risk of *Leishmania* spp. infection.

Keywords *Lu. Migonei*; ELISA, *Leishmania* INFECTION; VECTOR EXPOSURE

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P3-009: COINFECTIONS AND ZOONOTIC VISCERAL LEISHMANIASIS IN DOGS FROM A HIGH ENDEMIC AREA FROM RIO DE JANEIRO, BRAZIL

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In the state of Rio de Janeiro, zoonotic visceral leishmaniasis (ZVL) caused by the protozoan parasite *Leishmania* (*Leishmania*) *infantum*, which also can cause lethal infections in humans, is spreading to new areas. Therefore, the Brazilian Ministry of Health have implemented a national canine leishmaniasis control program. It is important to recognize that several other pathogens of dogs frequently originate subclinical infections in these animals, but clinical manifestations and possible transmission to the human population may occur due to the immunosuppression caused by ZVL. However, there are very few investigations on canine vector-borne diseases and endoparasitoses associated with ZVL. Within this context, the aim of this study was to evaluate the exposure of *Leishmania*-positive dogs living on a high endemic area in the State of Rio de Janeiro, Brazil, to other pathogens of zoonotic potential. In accordance with Brazilian guidelines for leishmaniasis control, blood samples were collected and 48 dogs were euthanized and subjected to necropsy. Antibodies against *Borrelia burgdorferi*, *Ehrlichia canis*/*E. ewingii*, *Anaplasma phagocytophilum*/*A. platys* as well as antigens against *Dirofilaria immitis* were investigated with the Snap® 4Dx® Plus test (IDEXX Laboratories, Westbrook, USA). The mucosa and contents of the small and large intestines were examined for

detection of helminths. Additionally, fecal samples were collected and submitted to coproparasitological techniques. Overall, 40% (19/48) dogs tested positive for at least one vector-borne pathogen. The pathogen *E. canis* was the most prevalent (40%;19/48), followed by *Anaplasma* spp. (10 %; 5/48), whereas 10% (5/48) of dogs showed coinfections with these two pathogens. All samples were negative for *Borrelia burgdorferi* and *Dirofilaria immitis*. A total of 31 % (15/48) of the dogs were positive for at least one intestinal helminth, being *Ancylostoma* spp. (19 %; 09/48) and *Dipylidium caninum* (14 %; 07/48) the parasites most frequently identified. The helminths *Toxocara canis* and *Trichuris vulpis* were detected in 8% (4/48) e 12% (6/48) of the dogs, respectively. Oocists of *Cryptosporidium* spp. and cysts of *Giardia* spp. was not detected in any of the faecal samples analysed. Based on our results, this study highlights a high prevalence of vector-borne pathogens and helminths with zoonotic potential in *Leishmania*-positive dogs in the examined area. The results also demonstrate that there is a need for tick, flea and helminth control in dogs infected with *L. infantum*. We encourage to reinforce the surveillance system to obtain a wider epidemiological control.

Keywords CANINE VISCERAL LEISHMANIASIS, HELMINTHS, RICKETTSIAS

P3-010: EXPLORATORY STUDY OF CANINE LEISHMANIASIS IN A FOCUS OF THE ANDEAN STATE TRUJILLO, VENEZUELA

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Visceral Leishmaniasis is a public health problem that in the last years has been of great importance in several endemic areas of Venezuela, being school age children the most susceptible. The canine is the reservoir of disease, which in addition to representing the source of infection for humans also suffers it. The Andean region in Venezuela is known to be a Cuteneous leishmaniasis endemic area and few reports has been associated in humans with the visceral disease. In the present work, an exploratory study of seroprevalence for canine visceral leishmaniasis was carried out in the Simón Bolívar neighborhood of the Sabana de Mendoza parish of Sucre municipality, Trujillo state, a population selected for presenting a recent history of human visceral leishmaniasis. A total population of 52 canines (65% male and 35% female) was evaluated, each of them underwent physical examination and blood sampling with informed consent of their respective owners. Epidemiological data were collected through a survey and direct observation of the study area. The climatological and sociodemographic data was georeferenced using QGIS software. The infection status of the canine population was determined using the ELISA-rk39 immunoassay and compared with the commercial rk39 Dipstick. The data show that 17,30% (9/52) of the evaluated canines presented specific antibodies against *Leishmania infantum*. There were no significant differences in variables such as age and sex regarding canine infection status. It was also demonstrated the high sensitivity of the ELISA vs the rapid test. The majority of infected dogs were classified as asymptomatic (61%) and of the symptomatic 20% showed multiple signs and symptoms including hepatosplenomegaly CONCLUSIONS: The results obtained suggest

a high prevalence in a well delimited area and a potential risk of acquiring the disease by both canines and humans, the fact that asymptomatic infected dogs represent the highest proportions imply an increased risk for which makes it necessary to implement measures of epidemiological control in the area.

Keywords CANINE LEISHMANIASIS, SEROPREVALENCE, EPIDEMIOLOGY

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P3-017: NEUROLOGICAL SIGNS CAUSED BY *Leishmania infantum* INFECTION – NEUROLEISH: AN EXPANDING REALITY

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Canine leishmaniasis (LCan) is caused by *Leishmania infantum* and transmitted by the bite of infected sandflies, mainly by the females of *Lutzomyia longipalpis* in the new world. It manifests as a systemic disease affecting several organs and systems, including the central nervous system in dogs. Three dogs of different ages and breeds, infected by *L. infantum* (confirmed by quantitative PCR of the bone marrow (BM)) and presenting neurological signs were treated. The neurological signs varied from ambulatory paraparesis, low or absent proprioceptive positioning, proprioceptive ataxia, urinary incontinence, delayed menace response, head-tilt, hyperesthesia on spinal palpation, and altered cutaneous truncus reflex. The total dilution of antibodies against *L. infantum*, measured by the indirect immunofluorescence technique (IIF), was 1:160 for all three animals. The ELISA test was reagent in two dogs. The parasite load in BM were 1.430, 108.000, and 34.785.064 DNA copies/µL, and all animals presented hyperproteinorachia between 285mg/dL to 394.2mg/dL. PCR for *L. infantum* in the cerebrospinal fluid (CSF) was positive in two animals, and in one of them, *Neospora caninum* and *Cryptococcus spp* were also found. In biochemical tests, all patients had hyperglobulinemia, and the blood count showed a hematocrit below the reference limit. The treatment using allopurinol (10mg/kg BID), miltefosine (2.2mg/kg for 30 days), and clindamycin (10 to 20mg/kg TID for 30 days) were common for all the animals. For two of them was administered dexamethasones (0.25mg/kg every 48 hours in three applications), while for the other prednisone (0.7mg/kg every 24 hours with dose reduction for 30 days until the end). Also, one animal received 16 applications of amphotericin B (1.7mg/kg) with an interval of three days between them. All animals improved the

neurological signs in about 45 days of treatment. Animals with neurological signs in endemic areas for *L. infantum* present should be investigated for neuro infection and other possible infectious etiologies. The differential diagnosis of LCan should be considered in these conditions, as it may be more prevalent than currently reported.

KEYWORDS: CANINE LEISHMANIASIS; DOGS; NEUROLEISH; DIFFERENTIAL DIAGNOSIS

P3-048: SEROPREVALENCE OF CANINE LEISHMANIASIS IN THE CITY OF SALTO, URUGUAY, 2020.

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Visceral leishmaniasis is a highly lethal zoonotic disease caused by protozoa of the genus *Leishmania* and transmitted by dipterans of the Phlebotominae family. This zoonosis is in expansion in Latin America, where the aetiological agent is *Leishmania infantum*, it is transmitted by the sandfly *Lutzomyia longipalpis* and its main reservoir is the domestic dog. In the year 2010, *Lu. longipalpis* was detected for the first time in Uruguay, in the cities of Bella Unión and Salto. In February 2015, the first cases of leishmaniasis were diagnosed in dogs from the city of Salto. In 2018, the first human case of visceral leishmaniasis was diagnosed also in Salto. To this day, the city had 7 human cases, one of whom passed away. The aim of this study was to estimate the seroprevalence of canine leishmaniasis in the city of Salto in order to be able to focus the control actions in the areas with the most intense transmission. A cross-sectional study was carried out from August 10 to 29, 2020. For the calculation of the sample size, a dog population of 58000 animals was estimated based on a dog population survey made at a national level. The expected prevalence was estimated to be 3%, a confidence level of 95% was used with a desired precision of 1%. The minimum number of dogs to be sampled was calculated to be 1097 dogs. To ensure the adequate geographical distribution of the animals, a random sampling of blocks in the city was carried out using the software QGIS v. 3.4.5. The mean number of dogs per block was estimated in 20. To achieve

the desired number of canines, 60 blocks were randomly selected, 5 more than necessary, anticipating some with few or no dogs. In the selected blocks, a house by house survey was made. In each house, blood from all the canines was obtained by puncture of the cephalic vein. The samples were centrifuged to obtain serum and subsequently the Kalazar Detect Canine rapid test (InBios International Inc.) was performed in the laboratory of Salto Departmental Health Office. The information was loaded into an electronic form for later analysis. From all the selected blocks, 57 had dogs, 3 had not. The mean number of dogs per block was 18.8. The number of dogs sampled was 1114, finding 47 positives with the test used. These were distributed in 30 of the 60 blocks. From all the infected canines, 91% were asymptomatic and 9% showed at least one sign of the disease. The general seroprevalence for canine leishmaniasis in the city of Salto was 4.20% (95% CI: 3.71-4.70) but varied considerably between regions. The regions with the highest seroprevalence correspond to the northwestern and southwestern areas of the city. Further investigation is needed to define the eco-epidemiological factors that drive the high prevalence of canine leishmaniasis in certain areas and its relation to human cases. Surveillance, prevention and control efforts must be reinforced, prioritizing the areas with the highest intensity of transmission.

Keywords CANINE LEISHMANIASIS; EPIDEMIOLOGY; SEROPREVALENCE; URUGUAY

P3-055: SPATIAL ASSOCIATION OF GENOTYPIC PROFILE OF *Leishmania infantum* WITH AREAS OF HIGH CONCENTRATION OF CASES OF CANINE OR HUMAN VISCERAL LEISHMANIASIS

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Although it has already been demonstrated the occurrence of different genomic profiles of *Leishmania infantum* in the Brazilian territory, it is still unclear its association with areas of concentration of cases of visceral leishmaniasis (VL), considering the environment where the main reservoir, the domestic dog, lives. Recent studies demonstrated Brazilian *L. infantum* strains presenting a 12Kb genomic deletion in chromosome 31 (chr31). This trait alters parasite biology, reflected by the reduced virulence *in vitro* and potentially the interplay with hosts, leading to a mild infection with a lower parasite load in the main reservoir. Bearing this in mind, infected dogs would remain under-detected by the current diagnostic methods recommended by the Brazilian VL control program and, as a consequence, remain in areas as (an unseen) source of infection, contributing to a higher concentration of canine (CVL) or human disease (HVL). This study aims to test the spatial association of the DEL genotypic profile of *L. infantum* with the historical concentration series of HVL (2011-2021) and CVL (2014-

2020) in Bauru and Votuporanga, Sao Paulo, Brazil. We evaluated 35 dogs living in urban areas, in which Dual-Path Platform (DPP), followed by enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR), confirmed the infection. We mapped samples using a Geographic Information System. A binary logistic regression model was fitted, in which we considered 1 for the presence of DEL strains and 0 for non-deleted strains (Non-DEL), heterozygosity or, possibly, a mixed population (HTZ or Mix). We applied the Kernel density estimator and extracted the value of the sample in the location of the *L. infantum* genotypes. The interpolation was classified into five classes of equal intervals (from low to high concentration), working as predictors. Overall, 42.8% (15/35) canines were infected by DEL, 42.8% (15/35) by Non-DEL, and 14.2% (5/35) by HTZ or Mix. We mapped 70 HVL and 369 CVL cases in Bauru, highly concentrated in the north and west of the city; and 498 HVL cases, and 1911 CVL cases in Votuporanga, highly concentrated in the north, west, and south of the city. The odds ratio (OR) for DEL strains was not spatially associated with the high concentration of HVL (OR 0.6463 CI 0.3422,1.2207); however, it was for the high concentration of CVL. DEL increased 112% the chances of having the CVL in areas of high concentration of CVL cases (OR 1.1248 CI 0.7019, 1.8025); therefore, our preliminary findings suggest that DEL strains can be associated with areas of high concentration of cases, although no statistical significance was observed. While we are not yet able to increase the samples for further analysis, it is important to keep in mind that the genomic profile of *L. infantum* may have different roles in the local space where VL is endemic.

Keywords SPATIAL ANALYSIS; HIGH-CONCENTRATION; SPACE; GENOMIC PROFILE; DOGS; VISCERAL LEISHMANIASIS

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P4-001: SEROCONVERSION ASSESSMENT OF COMMERCIALIZED SEROLOGICAL TESTS AND THOSE RECOMMENDED BY THE MINISTRY OF HEALTH OF BRAZIL FOR THE DIAGNOSIS OF DOGS VACCINATED WITH LEISH-TEC®

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Different types of diagnostic tests are used to detect canine visceral leishmaniasis (CVL). In small animal clinical routine and government programs, serological tests are considered the tests of election. To indicate the use of anti-leishmaniasis vaccines, it is recommended that dogs be tested before starting a vaccination protocol. The evidences reveal a possible influence of the humoral immune response on serological tests, influencing the distinction between vaccinated and truly infected animals. In Brazil, the only commercial vaccine registered for individual use is Leish-Tec® (CEVA Saúde Animal®). According to the producer, the recombinant A2 protein used in immunobiologicals induces a cellular immune response in challenged animals, presenting a lower antibody load and stimulus. Considering the need for serological tests that do not produce reactive results influenced by the immune response of the humoral vaccine, the present study evaluated the possible seroconversion of healthy dogs immunized with the Leish-Tec® vaccine. This is a prospective study conducted in nine dogs from the Brazilian Air Force. As inclusion criteria, healthy dogs, older than four months, asymptomatic and with negative serological and molecular tests were defined. The serological screening samples used were the commercial rapid immunochromatographic test produced by the Alere® laboratory (rK39 protein) and the TR DPP® test (rK28 protein), and the confirmatory ELISA (Enzyme Linked Immuno Sorbent Assay), the latter two recommended by the Brazilian Visceral

Leishmaniasis Surveillance and Control Program. For the Real-Time Polymerase Chain Reaction (qPCR), a bone marrow sample was used. The animals were inspected for 120 days, and collars was placed in all of them in order to minimize possible exposure to the vector. Out of the nine animals, seven met the inclusion criteria and were observed during the determined period. The dogs were vaccinated according to the producer's recommended methodology. Eligible animals received only one booster dose, since the group had already been vaccinated 12 months before. On the 120 th day, venipunctures were performed for serological tests, and bone marrow aspirate for molecular examination. Samples 3, 6 and 9 (27%) were reagent on the TR-DPP®, and on the Alere® test only one (9%), with sample 3 being reactive in both laboratories. When submitted to the confirmatory ELISA test, all 3 samples presented non-reactive results. All bone marrow samples processed in qPCR also showed negative results. It is concluded that the use of immunomatographic tests can influence the distinction between vaccinated and naturally infected dogs, recommending the association of other diagnostic techniques such as ELISA and qPCR in the diagnosis of dogs with a history of Leish-Tec® immunoprophylaxis. However, a larger number of dogs need to be evaluated in order to make apropriate inferences from the results found.

keywords CANINE VISCERAL LEISHMANIASIS; VACCINE; DIAGNOSIS; SEROCONVERSION

P4-002: SUPPLEMENTATION WITH NUTRACEUTICO DEFENSYN® AND ITS EFFECTS ON CLINICAL IMPROVEMENT OF DOGS WITH VISCERAL LEISHMANIASIS.

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Drugs with leishmanicide and leishmaniostatic activities, as well as those with immunomodulatory components, had been used in the therapy of Canine Visceral Leishmaniasis (CVL). It is known that CVL chemotherapy is still a challenge, due to the lack of effective treatment that can kill the parasite. The supplementation with nutraceuticals may be a potent adjunct to elicit a good immune response additionally to the treatment of VL in dogs. β-glucans are polysaccharides, found in yeasts, which have immunomodulatory action reported for years. The occurrence of systemic lesions of CVL is directly related to a poor host's immune response and disease progression. This study aimed to evaluate the supplementation using Defensyn® associated com allopurinol in dogs naturally infected with *Leishmania infantum* and its effect on the clinical improvement of these animals. This study used 25 dogs with infection confirmed by serological and or parasitological tests. The animals were divided into three groups, Group 1 (G1) with nine animals which received Allopurinol at a dosage of 15mg/kg once daily, supplemented with Defensyn® with 3g/5kg once daily, Group 2 (G2) with nine animals who received only Allopurinol at the dosage already described, and Group 3 (G3) with seven animals that did not receive therapy (control). The study was conducted for 60 days, evaluating parameters before (D0) and at the end of the trial (D60), as clinical evaluation using a score referring to symptomatology, laboratory tests such as hematology, serum biochemistry (renal and hepatic function, proteins and fractions, albumin globulin ratio), serology (ELISA and IFAT),

parasitological (direct medullary, lymph node and skin) and q-PCR. Statistical analysis was performed using GraphPad Prism 8.0 software (GraphPad Prism Inc., San Diego, CA) and the significance level was considered for $p<0.05$. In the individual comparison of each group (D0 and D60), the score of group 1 showed a greater reduction in clinical signs ($P<0.01$), followed by group 2 ($P<0.05$), while group 3 did not show any clinical improvement. In the evaluation between groups, there was a significant difference between group 1 and group 3, on Day 60. The results of hemogram, serology, and biochemistry showed no significant difference, with values within the normal range, except for alkaline phosphatase, which increased in groups 2 and 3 after the end of the 60 days of follow-up ($P<0.05$). The parasitic load of group 1 remained constant after 60 days of the experiment, while groups 2 and 3 showed an increase in parasitemia ($P<0.05$). Although there was no significant reduction in parasitic load, the clinical signs of the group supplemented with Defensyn® showed a good clinical improvement in the evaluated times, the non-supplemented groups showed a significantly higher parasitic load at the end of the trial. Supplementation with Defensyn collaborates with therapy in the clinical improvement of dogs with visceral leishmaniasis.
Keywords KALA-AZAR; CANINE; SYMPTOMOLOGY; SUPPLEMENT; THERAPY

Financing Konig Brazil.

P4-003: SEROCONVERSION AND LEVELS OF ANTI-LEISHMANIA ANTIBODIES COMPARED WITH THE SYMPTOMATOLOGY OF DOGS INFECTED EXPERIMENTALLY WITH *Leishmania infantum*

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Visceral leishmaniasis (VL) is a zoonotic and endemic parasitic disease in some regions of Brazil, which has the dog as the main reservoir, and the diagnosis in this species is based on the detection of circulating anti-Leishmania antibodies against parasite antigens. The objective of this work was to evaluate seroconversion with serial dilution of sera from dogs experimentally infected with *L. infantum* to determine the moment the animal begins to produce anti-Leishmania antibodies circulating after infection and its titration from the moment of seroconversion, associating with the clinical status and disease progression. Sera from 11 beagle dogs experimentally infected with *L. infantum* were used. Serum samples were analyzed 8 times (D0, D30, D60, D90, D120, D180, D210, and D240). The clinical profile was analyzed by scores and recorded in records, having as main parameters the size of lymph nodes, skin involvement, weight loss, and ocular lesions. The detection of anti-Leishmania IgG antibodies was performed using the ELISA kit (EIE-Bio-Manguinhos). The seroconversion time after the experimental infection was determined and the titration of the animals was performed from the dilution of 1:100, as recommended by the kit manufacturer. The statistical analysis was performed using the GraphPad Prism Software version 8.0 and the significance level was considered for $p<0.05$. The results showed that clinical signs began to appear 120 days after infection and increased up to 240 days, with the enlargement of pair of lymph nodes, onychogryphosis, weight loss, and ulcerative lesions in the skin being the main clinical findings. 36% (4/11) of the animals were positive in serology after 120 days of experimental infection and reactivity

increased reaching 91% (10/11) in the last period analyzed. In the titration analysis, it was observed that the antibodies levels of the animals increased over time and that, after 180 days, 36.36% of the evaluated animals presented titration ranging from 1:100 to 1:3200. After 210 days of infection, 63.63% of the evaluated animals had high antibody titers, reaching a value of 1:6400, a value that remained until 240 days after infection. This study showed that, in experimentally infected beagle dogs, seroconversion occurred 120 days after infection, and that, as time progressed, the increase in antibody titers accompanied the clinical progression of the disease in this experimental model.

Keywords TITRATION; CLINICAL SIGNS; KALA-ZAR; IMMUNOGLOBULINS; LEISHMANIASIS; CANINE

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P4-005: MYELOPROLIFERATIVE DISORDERS ASSOCIATED WITH CANINE LEISHMANIASIS STAGING

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The immune response in the various lymphoid compartments is a useful tool in the study of the progression of canine leishmaniasis (CanL). The bone marrow (B.M.), the primary lymphoid organ, is responsible for maintaining the cells of the erythroid, myeloid, lymphoid and platelet lines. It is one of the most favored sites for the multiplication of *Leishmania spp.* amastigote forms that can lead to ultrastructural changes in the previously mentioned organ, according to the progression of the disease. In Lcan the B.M. it is usually evaluated only on its parasitism. This study aimed to associate the findings of B.M parasitism and myelogram to the clinical staging of CanL proposed by the Study Group on Animal Leishmaniasis. Twenty-three adult dogs went to clinical evaluation and had biological samples collected to investigate CanL *in vivo*. Serological tests (ELISA), B.M. molecular investigation (PCR) and B.M. parasitological analyzes were performed (cytology) in addition to blood count and serum biochemistry. We found that 18/23 (78.2%) dogs were serologically positive. Of this group, molecular diagnosis were confirmed in 8/18 (44.4%) and the parasitological diagnosis in 3/18 (16.7%). Myelogram showed an increase in the myeloid: erythroid ratio (M: E) in 11/18 (61.1%). Anemia was seen in

5/18 (27.8%). The dogs were staged as stage I 4/18 (22.2%), stage II 13/18 (72.2%) and stage III 1/18 (5.6%). The investigation of the parasitism of the B.M. in human leishmaniasis and CanL is well elucidated, but myeloid alterations associated with medullary parasitism, peripheral hematological alterations and clinical progression are poorly described in current literature. The clinical progression of CanL has a direct correlation with changes in hematopoietic and hematological parameters. In this study, we investigated for the first time the presence of alterations in the M:E ratio in dogs in the early stage of CanL (stage I). According to our findings, alterations in the M:E ratio may occur at an early stage, even before the peripheral blood reflex - anemia. In human leishmaniasis myeloproliferative disorders are correlated with worse prognosis and lower therapeutic response. These findings suggest that the myelogram is an early biomarker of myelopathies associated with early staging of CanL.

Keywords MYELODYSPLASIA; BONE MARROW; CANINE LEISHMANIASIS; STAGING

P4-007: CANINE VISCERAL LEISHMANIASIS: ELISA IN HOUSE PERFORMANCE USING NATIVE STRAIN FROM THE 2015 OUTBREAK IN URUGUAY

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Visceral leishmaniasis (VL) is a zoonotic disease caused by flagellated protozoa of the genus *Leishmania* and transmitted by sand flies belonging to the Phlebotominae subfamily with *Lutzomyia longipalpis* as the main vectors. VL affects humans and canids, the latter being the main reservoir of the parasite. This zoonosis has been endemic in northeastern Brazil for several centuries, but it has been recently expanding to southern areas of the South American continent: the right environmental conditions, the presence of competent sand fly vectors, and the constant appearance of new cases of canine and human leishmaniasis in border countries have made Uruguay susceptible to VL transmission. The purpose of this job was to evaluate the performance of an ELISA using our own antigens and compare it with a commercial kit (Bio-Manguinhos), which is being used by the Public Health Ministry of Brasil as a confirmatory test for canine VL. Up to 205 dogs older than 6 months were selected during the active surveillance for CVL performed by the Ministry of Public Health, from July to December 2019, age, race, sex, clinical symptoms, sleeping site and city were registered and serum samples collected. To develop the ELISA in house we obtained antigen from promastigotes from a stationary phase culture of *Leishmania infantum* (MCAN / UY / 2015 / bCH11) that was obtained from spleen of VL positive dogs during the 2015 outbreak at Arenitas Blancas. The antigen elaboration, plate sensitization and work solutions were made following the

protocol of the Instituto FIOCRUZ (Paraná, Brasil), a reference Laboratory at VL diagnosis. The obtained results for the ELISA in house, having the commercial ELISA (Bio-Manguinhos) as a reference with a confidence interval of 95% and an estimated prevalence of 32%, are: 100% sensitivity and 88,7% specificity for symptomatic, and 85,7% sensitivity and 94,4% specificity for asymptomatic. The positive predictive value was 83.3% and the negative predictive value was 98.5%. Concordance among the two ELISA test was found to be almost perfect by determination of the kappa index. Other jobs obtained similar results when they evaluated the sensitivity of and crude antigen-based ELISA versus a recombinant antigen-based ELISA (technology used on ELISA Bio-Manguinhos), obtaining sensitivities of 94,1% and 90,6%, respectively. This could be happening on our study, highlighting that ELISA Bio-Manguinhos uses recombinant antigen from *Leishmania major*. Species-specific variations could also influence our results in favor of ELISA in house. In conclusion, the ELISA in house has a high concordance with the ELISA Bio-Manguinhos, which is already validated. This result motivates us to embark on the validation process looking to propose it as the validation technique following the example of Brasil.

Keywords *L. infantum*; SEROLOGICAL; DIAGNOSIS; ELISA IN HOUSE

P4-009: IMPORTANCE OF THE METHOD USED TO DETERMINE THE COMPOSITION OF UROLITHS IN DOGS NATURALLY INFECTED WITH *Leishmania infantum* – A RETROSPECTIVE STUDY FROM 2005-2021.

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The use of allopurinol in canine leishmaniosis (LCan) treatment is frequent reported as a cause of urolithiasis. However, uroliths composition has not uniformly recorded in analyzes performed in different laboratories. In this sense, data from patient's chart attended in Santo Agostinho Hospital Veterinário was accessed in order to evaluate the occurrence and composition nature of uroliths found in 33 dogs naturally infected by *Leishmania infantum* in the period from 2005 to 2021. This retrospective study shown that 66.7% (22/33) of the dogs were male and 33.3% (11/33) were female; and uroliths of the highest incidence was urate (39.4% - 13/33), followed by xanthine (21.2% - 7/33). Besides, calculi of other natures, one of struvite and the another one of mixed composition, were identified in two infected males, that were not been treated with allopurinol. Related to breed predisposition, the uroliths were more frequent detected in 9/33 mongrel dogs (27.3%) and in 5/33 Labrador Retrievers (15.2%). Taken all the results together was observed that males presented more frequently urate uroliths (9/22 – 40.9%) and xanthine (6/22 – 27.3%); and in females struvite (4/11 – 36.4%) especially in Schnauzers (3/4 – 75%). Moreover, identification of xanthine calculi varied according to the methodology employed in its analysis suggesting that the technique used in each laboratory can directly influence in xanthine uroliths identification. The physical-chemical methodology for uroliths analysis, which characterizes the total chemical composition of the stone and its macroscopic characteristics, showed less precision when compared to the techniques of energy dispersive spectroscopy, optical crystallography or

infrared spectroscopy. These ones provide the stone layers composition with greater precision increasing sensitivity in identify the presence of xanthine in the stones composition. In this way, this study suggested that the choice of methodology for stone identification direct influences in it correct categorization, enabling the clinician establishing the correct therapeutic management of patients undergoing continuous treatment with allopurinol.

Keywords DOG, LEISHMANIASIS, ALLOPURINOL, UROLITHS

P4-010: OCCURRENCE OF INTESTINAL HELMINTHS IN DOGS INFECTED WITH *Leishmania* (*Leishmania*) *infantum*: ASSOCIATION WITH CLINICAL SIGNS AND HISTOLOGICAL CHANGES

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Zoonotic visceral leishmaniasis (ZVL) is a widely distributed infectious disease and infected dogs are considered the main domestic reservoirs of the parasite. The association of intestinal helminths with ZLV in dogs is poorly understood. The objective of this study were to evaluate the occurrence of intestinal helminths in dogs and to compare clinical signs and histological alterations of those coinfected by intestinal helminths with those monoinfected by *Leishmania* (*Leishmania*) *infantum*. The sample was composed of 41 dogs, which were euthanized and necropsied. Fecal samples were collected and submitted to coproparasitological techniques. Additionally, the mucosa and the contents of the small and large intestines were examined for detection of helminths. The fecal samples were analyzed by centrifugation in 1.26 g/ml sucrose solution and spontaneous sedimentation. The helminths found in the examination of the intestinal mucosa and content were fixed and identified. A positive frequency of 53.6% was observed for helminths. *Ancylostoma* spp., *Dipylidium caninum*, *Trichuris vulpis* and *Toxocara canis* were identified. In the monoinfected group, the frequencies of dogs with many clinical signs, few clinical signs, and no clinical signs were 52.6%, 36.8%, and 10.5%, respectively. In the coinfected group, the frequencies of dogs with many clinical signs, few

clinical signs, and no clinical signs were 77.2%, 18.1%, and 4.5%, respectively. The frequency of intestinal inflammation in the in the monoinfected group was 31% and in the coinfected group was 69%. The higher frequency of clinical signs and intestinal inflammation in coinfected dogs suggest that coinfection by intestinal helminths contributes to the worsening of ZVL in dogs, with a potential risk of zoonotic transmission of *L. infantum* and of some helminths. For humans and dogs in the studied area.

Keywords LEISHMANIASIS; HELMINTHIASIS; DOMESTIC RESERVOIR; HISTOLOGY

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P4-085: EVALUATION OF THE FIRST DOG COLLARING CYCLE IN BRAZIL

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In Brazil, the Ministry of Health is responsible for developing national guidelines for the control of visceral leishmaniasis (VL). Recently, the National Visceral Leishmaniasis Surveillance and Control Program (PVC-LV) has incorporated the use of collars impregnated with 4% deltamethrin in the canine population as an additional tool to prevention and control measures. The PVC-LV carried out the categorization of the municipalities in the country with transmission of VL based on risk indicators, and defined the priorities for the application of the intervention. According to the latest classification, about 15% of municipalities with transmission are considered priority and account for 70% of human VL case records. In the Southeast region, the municipality of Montes Claros, Minas Gerais, is considered endemic for the disease and with intense transmission. This municipality started the implementation of collaring in 2021 and established priority areas based on indicators recommended by, among them, the cumulative incidence rate of VL and at least one of the following indicators: canine density, canine prevalence and/or socioeconomic vulnerability. This work aims to describe the characteristics of the baseline population of a cohort of dogs followed up to evaluate operational aspects of collaring with collars impregnated with DM4% in a municipality with high transmissibility. Method: For sample selection, resident dogs from eight local work areas (ATL) that met the eligibility criteria for collaring were included. For sample

calculation, a confidence level of 95% was defined, a sampling error of 5%, and an estimated canine prevalence of 8%. As inclusion criteria, only domiciled or semi-domiciled dogs diagnosed as non-reactive in the serological tests were collared. To monitor the population of dogs in the study, the tutors were contacted after 90 days of collaring to verify the health status of the animal and the presence/absence of the collar on the dog. Results: 299 dogs were evenly distributed in the 8 ATLs. 75% of the animals were medium and small, 55% females. The most frequent age range was 1-3 years (36%), and 91% were domiciled. On D90, 17.7% (53/299) of the dogs in the sample were no longer wearing the collar. The main causes of loss were death from other causes (20.5%) and removal of the collar by the dog or guardian (15.5%) or development of an allergic manifestation (28.9%). On average, the animals lost their collars after 44 days of collaring. Discussion: the preliminary results of this study show a high loss of collars in the first 90 days of collaring, mainly due to animal deaths. This result is in agreement with literature data that estimated a loss between 27-56%. The results of a prospective study will contribute with information for the planning of this intervention and to evaluate the impact of the loss of collars in the effectiveness of the actions of the VL control program and the necessary actions to improve the implementation of this strategy.

Keywords CANINE VISCERAL LEISHMANIASIS; VACCINE; COLLARS

5.2. DIAGNOSIS-TREATMENT AND RESISTANCE-CLINIC

P1-013: LANG: ISOTHERMAL AMPLIFICATION COUPLED TO GOLD-BASED NANOROD NANOSENSORS FOR FAST AND DIRECT DETECTION OF *Leishmania* DNA

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Real-time polymerase chain reaction (q-PCR) is an important technique used in the differential molecular diagnosis of leishmaniasis by detecting DNA of *Leishamania* parasites. However, some drawback limit its use on a large scale and in Point-of-Care (PoC) applications. Thus, the loop-mediated isothermal amplification (LAMP) technique proved to be a viable and promising alternative as molecular diagnostic tool, easy to perform at low cost being affordable and compatible with PoC format. The results obtained by this technique are usually read through association of different non direct techniques as fluorometry, turbidimetry, fluorescence and colorimetry with pH indicators. However, such reading methods need to be improved in order to increase reliability, sensitivity and specificity. In this regard, we developed biosensors using gold nanorods (GNRs) functionalized with specific sequences, complementary to the amplicons derived from LAMP reaction. The tool termed here iAnG (isothermal amplification and nano gold) was designed to detect DNA from *Leishmania amazonensis*, *L. braziliensis* and *L. infantum*. The colorimetric system output can also be measured by UV-Vis absorption and dynamic light scattering, being extremely sensitive enabling LAMP amplicon detection after as short as 15

min amplification reaction (instead of 40 to 60 min standard LAMP reaction). Obtained results showed a great sensors fast response, with emphasis on *L. infantum*. Sensors for *L.braziliensis* and *L.amazonensis* needed more volume of reaction product to confirm detection, suggesting that the amount of amplicom interferes in mesure with the chosen output. Here we show a proof of concept, made firstly with DNA extracted from *Leishmania* culture and that showed for the first time, direct amplicon identification obtained by LAMP reaction of the different *Leishmania* species through biosensors based on GNRs. We are still working on complementary tests to improve and validate this thechnique using clinical samples derived from visceral and cutaneous leishmaniasis. The iAnG system can improve LAMP-based diagnostics providing higher sensitivity, and specificity in a cheaper and fast way, by reducing consumables amount and reaction time. iAnG provides an easy, effective and accurate diagnostic tool that can be used to control leishmaniases specially in limited resources regions.

Keywords LAMP; MOLECULAR DIAGNOSTIC; LEISHMANIASIS; BIOSSENSORS; GOLD NANORODS

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P1-015: ACCURACY OF MOLECULAR, SEROLOGICAL AND IMMUNOHISTOCHEMICAL TESTS FOR THE DIAGNOSIS OF LEISHMANIASIS WITH MUCOSAL INVOLVEMENT

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Leishmaniasis with mucous involvement is considered one of the most neglected forms of leishmaniasis. In Brazil, mucosal lesions are reported in about 6% of the cutaneous leishmaniasis cases. Mucosal involvement poses additional challenges to the diagnosis, either because of the difficulty in accessing mucosal surfaces or because of the scarcity of parasites in these sites. In this context, this study aims to describe the accuracy of a new monoclonal antibody for immunohistochemistry, two real-time polymerase chain reaction (PCR) tests (SYBER detection system for kDNA target and TaqMan detection system for SSU rRNA target), a conventional PCR technique based on hsp70 target, and five serological tests based on immunoassay platform, namely: Viercell® test – Leishmania ELISA IgG+IgM, Virion/Serion test – SERION® ELISA classic Leishmania IgG, in house ELISA based on soluble antigen of *L. braziliensis* and two ELISAs using the recombinant protein antigens β-TUBULINA (*L. braziliensis*) and LiHyV (*L. infantum).* Between April 2017 and September 2020, 124 patients with mucosal manifestation and leishmaniasis suspicion were recruited at a referral centre in Brazil, 100 of which were confirmed with the disease by parasitological or molecular criteria (qualitative PCR with kinetoplast DNA

target). The highest sensitivities were observed for real-time PCR tests (94.2% for qPCR/Syber and 87% for qPCR/TaqMan), both with specificity around 85%, with no statistical difference between them. Among the immunoassays, moderate sensitivity was observed for two tests: 71.7% for in-house ELISA based on *L. braziliensis* antigen and 62.3% for commercial ELISA using *L. infantum* antigen. The sensitivity of immunohistochemistry was 45%, with specificity of 90%. Among the molecular tests, the two real-time techniques Syber qPCR and TaqMan kDNA exhibited the highest performance. However, these techniques require high technological facilities and specialized professionals, which makes their use outside large urban centers unfeasible. Despite ELISA VIRCELL® and ELISA based on total *L. braziliensis antigens* exhibited a highest sensitivity compared to the other immunological tests, their accuracy rates are still insufficient to routine use in clinical practice. In summary, our results confirm molecular tests as the main strategy for the diagnosis of leishmaniasis with mucosal involvement. So far, serological tests have not been sufficiently accurate to compose the investigation algorithms and immunohistochemistry plays an adjuvant role in the histological examination.

Keywords LEISHMANIASIS; MUCOUS INVOLVEMENT; DIAGNOSIS; ACCURACY

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P1-016: NEW ANTI-β-GB PROTEIN MONOCLONAL ANTIBODY-BASED IMMUNOHISTOCHEMISTRY FOR CUTANEOUS LEISHMANIASIS DIAGNOSIS

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Cutaneous leishmaniasis (CL) is a dermatological disease caused by flagellated parasites and constitutes a serious public health problem in the Americas, Asia, Europe, and Africa. A great number of human CL-cases caused by different species of *Leishmania* are recorded in Brazil annually. Laboratory diagnosis is done by parasitological techniques and/or molecular techniques when they are available at a reference center. Consequently, about 20% of human CL-cases are treated without confirmation by laboratory diagnosis. Given this scenario, we developed an immunohistochemistry (IHC) technique for CL-diagnosis based on an anti-*Leishmania* guanine nucleotide-binding protein beta-like protein monoclonal antibody (mAb anti-β-GB Protein). The *Leishmania* (*Viannia*) *braziliensis* β-GB Protein sequence (A4HGX7_LEIBR) was submitted to epitope prediction and a minigen containing three antigenic regions was constructed and inserted in pET28a. Recombinant *Leishmania*-β-GB Protein was produced and used as an immunogen for mAb production through somatic hybridization. The capacity of mAb anti-β-GB Protein to recognize native *Leishmania*-β-GB Protein was assayed by Western blotting using soluble antigens of *Leishmania (Leishmania) amazonensis, L. braziliensis* and *Leishmania* (*Viannia*) *guyanensis*. The applicability of mAb anti-β-GB Protein in the immunohistochemistry technique was also evaluated in histologic sections of skin biopsies from hamsters experimentally infected with *L. amazonensis*, *L. braziliensis* and *L. guyanensis*. Antigen-antibody reaction

was revealed with polymers conjugated with the enzymes horseradish peroxidase (IHC-HRP) and alkaline phosphatase (IHC-AP), both biotin-free detection systems. mAb anti-β-GB Protein recognized native *Leishmania*-β-GB Protein in *L. amazonensis*, *L. braziliensis* and *L. guyanensis* soluble antigens. Furthermore, IHC-HRP and IHC-AP easily detected amastigotes from *L. amazonensis*, *L. braziliensis* and *L. guyanensis* in histological sections from hamsters experimentally infected with these parasites, but IHC-AP presented higher chromatic contrast for amastigote visualization. We conclude that IHC-AP demonstrated greater adequacy for CL diagnosis and will be validated in a panel of histological sections from patients with CL and other dermatological diseases.

Keywords CUTANEOUS LEISHMANIASIS; IMMUNOHISTOCHEMISTRY; DIAGNOSIS; MONOCLONAL ANTIBODY; LEISHMANIA-β-GB PROTEIN

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P1-018: SPLICED LEADER RNA DETECTION AS A PAN-*Leishmania* DIAGNOSTIC METHOD AND PRACTICAL INTEGRATION WITH STANDARD KINETOPLAST DNA ASSAYS

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Diagnosis of leishmaniasis relies on microscopic, immunochromatographic and molecular methods. Most PCR-based assays amplify minicircle kinetoplast DNA (kDNA) which is present in high copy number and favors a high sensitivity. However, kDNA lacks the ability to discriminate viable *Leishmania* parasites and genetic heterogeneity hinders universal *Leishmania* detection. Recently, we developed a *pan-Leishmania* assay that targets the conserved spliced-leader RNA (SL-RNA). This short 39-bp noncoding sequence is attached during trans-splicing to the 5′-end of all mature nuclear mRNAs, a process required for RNA processing, transcript stability, and initiation of translation. This assay was evaluated against eight Old and New World *Leishmania* species, demonstrating specific detection and equal analytical sensitivities, irrespective of the species. The assay was validated on tissue samples of infected hamsters, promastigote spiked human blood and blood samples from visceral leishmaniasis (VL) patients with a sensitivity equal to the kDNA assay. The SL-RNA qPCR also proved applicable on laboratory *L. major* infected sand flies (*Lutzomyia longipalpis*) and field collected sand flies (*Phlebotomus pedifer*) harboring *L. aethiopica* in combination with a crude extraction method, allowing high-throughput screening. In a next step, various sampling, stabilization and extraction

methods have been compared to enable downstream SL-RNA and kDNA detection on a single patient's blood sample for comparison. Nucleic acid stabilization prior to storage showed to significantly improve detection. Laboratory assays on intramacrophage amastigotes have been performed to comparatively assess the half-life of the molecular targets after treatment with various reference drugs and antileishmanial leads. The rate of decay following treatment was faster for SL-RNA than kDNA, more reliably following the parasite killing kinetics observed by microscopy. This indicates that SL-RNA detection is potentially useful in clinical studies in which the detection of viable parasites is pivotal to assess parasitological cure and predict poor treatment outcome or relapse. Therefore, we are currently translating our experimental findings through assessment of the decay of kDNA and SL-RNA over time in blood of VL patients from Arba Minch General Hospital in Ethiopia during 18 days of combination therapy with paromomycin and SSG. Overall, our work demonstrates a broad range of applications and characteristics of the SL-RNA assay, and provides a number of practical recommendations that improve molecular detection of *Leishmania* RNA and DNA from a single blood sample.

Keywords SPLICED LEADER RNA; LEISHMANIASIS; STABILIZATION; HALF-LIFE; VIABILITY MARKER

P1-019: DEVELOPMENT AND VALIDATION OF A READY-TO-USE GEL FORM NESTED PCR AND A REAL TIME PCR FOR DIAGNOSIS OF LEISHMANIASIS

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Leishmaniasis is an endemic parasitic disease in 98 countries. In Spain, it is a zoonosis caused by *Leishmania infantum*, its distribution is focal, with a reported average annual incidence of 0.45 cases/100,000 inhabitants, although in recent years, in the southwestern area of the Community of Madrid, this incidence rose at 43.5 cases/100,000 inhabitants. The predominant clinical manifestations are the cutaneous and visceral forms. Parasitological, serological, and molecular tests are useful for early confirmation of clinical suspicion and timely treatment. Parasite detection is considered the gold standard, and polymerase chain reaction (PCR) showed the greatest sensitivity and specificity. Therefore, at the WHO Collaborating Center for Leishmaniasis (WHOCCLeish), routine diagnostic tests are based on PCR (Ln-PCR) and culture. In this work, we describe the development and validation of a gel format of the Ln-PCR (LeishGelPCR), and its version in real-time PCR (Leish-qPCR). Ln-PCR is a nested in-house PCR that is performed in two rounds of reaction. LeishGelPCR is a ready-to-use system, only the DNA and water must be added to reach the final reaction volume, it is also carried out in two reaction rounds. Leish-qPCR is a multiplex real-time PCR that allows the simultaneous detection of *Leishmania* and the internal control of the reaction. For the development and determination of the analytical sensitivity of the new formats, 1/10

serial dilutions of DNA from *L. infantum* cultures were made. In the validation, 200 clinical samples from the WHOCCLeish collection were analyzed. Specificity was determined by analyzing samples from patients with other parasites and from healthy individuals. In this study 92 and 85 of 94 and 87 clinical samples were positive by LeishGelPCR and Leish-qPCR, respectively, showing a sensitivity of 98% in both approaches. The specificity of LeishGelPCR was 100% (n = 105). Two out of 105 negative samples were positive by Leish-qPCR, so specificity was 98%. The analytical sensitivity (limit of detection) of both protocols was similar (0.5 and 0.1 parasites/reaction). The parasite load was similar in VL and CL cases, however significant differences were observed according to matrix sample. In summary, the original Ln-PCR, gel-form system, and qPCR can be used interchangeably. The gel-form allows guaranteeing the homogeneity of the PCR reactions, avoiding differences between days, operators, and laboratories. Both the gel-form and qPCR reduce reagent handling and therefore also reduce the likelihood of contamination.

Keywords LEISHMANIASIS; MOLECULAR DIAGNOSIS; SENSITIVITY; SPECIFICITY

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P1-020: LINEAR EPITOPE MAPPING OF *Leishmania infantum* RECOMBINANT PROTEINS THROUGH MICROARRAY ANALYSIS TO IMPROVE THE DIAGNOSIS OF VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) has expanded worldwide, and, in Brazil, it has shown growing incidence in urban areas. Serological diagnosis is the main diagnostic method used to detect *Leishmania infantum* infection in Brazil. The diagnostic protocol recommended by the Brazilian Ministry of Health is a screening assay using a rapid test in dogs (the main urban reservoir of the disease) and humans, followed by a confirmatory ELISA test for dogs and an immunofluorescence assay for humans. Despite the low cost and the ease of performance, the diagnostic tests recommended for VL diagnosis present accuracy problems and cross-reactivity with other species of the Trypanosomatidae family. Therefore, more immunogenic and specific antigens should be selected to avoid cross-reactivity and to enable the detection of more seropositive patients. Therefore, to improve the accuracy of diagnostic tests, our group identified three recombinant proteins of *L. infantum* (rLci1a, rLci2b, and rLci5) in ELISA and rapid tests, which presented higher sensitivity and specificity than the tests recommended by the Brazilian Ministry of Health. The use of these proteins also showed high accuracy values (84% - 92%). However, there was cross-reactivity with *T. cruzi* (35% for rLci5), *Babesia* sp. or *Ehrlichia* sp. (27% for rLci1, 9% rLci2, and 18% for rLci5). Thus, to improve the accuracy of these proteins in VL diagnosis, bioinformatics analyzes (BLAST-p, Clustal Omega) and peptide

microarray studies were carried out in order to identify exclusive and cross-reactive epitopes in *L. infantum, T. cruzi, Ehrlichia canis* and *Babesia canis* (species commonly found in coinfections), and select those immunogenic only for *Leishmania* for use in a diagnostic test. After in silico evaluation, similarity matrices were constructed, high similarities with *T. cruzi* (92%), *E. canis* (47%), and *Babesia* species (64%-74%) were identified for rLc1a. While rLci5 and rLci2b revealed only similarities to proteins within the *Leishmania* genus. An ELISA was conducted with proteins in their original conformation and after denaturation at 80ºC for 20 minutes to estimate the reactivity to linear epitopes of each protein. Protein rLci2 presented 86% sensitivity, 86% specificity, and 64%, 87%, and 0% cross-reactivity to *B. canis*, *E. canis*, and *T. cruzi*, respectively. Denaturated rLci2 presented the same sensitivity, higher specificity (100%), and less cross-reactivity (45%, 75%, and 0% to *B. canis*, *E. canis*, and *T. cruzi*, respectively). Protein rLci5 presented 86% of sensitivity, 100% of specificity, and 45%, 67%, and 0% of cross-reactivity to *B. canis, E. canis*, and *T. cruzi*. Denaturated rLci5 presented lower sensitivity (43%), same specificity, and lower cross-reactivity (27%, 50%, and 0% specificity to *B. canis, E. canis*, and *T. cruzi*, respectively). In addition, linear epitopes recognized by anti-*Leishmania* antibodies were mapped using peptide microarray assays. Seven regions of interest from all three proteins were identified. Candidate epitopes for an accurate diagnosis can be identified from these regions in order to improve the specificity and maintain or improve the sensitivity of VL diagnosis.

Keywords *Leishmania*; RECOMBINANT PROTEINS; EPITOPE MAPPING

Financing PROEX - CAPES, PROEP IGM-FIOCRUZ N° 01/2020 and CNPq

P1-021: PERFORMANCE EVALUATION OF DIRECT MICROSCOPIC EXAMINATION FOR *Leishmania* spp. DETECTION IN A PUBLIC HEALTH REFERENCE LABORATORY IN COLOMBIA

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Cutaneous leishmaniasis (CL) is the most common clinical manifestation of the disease in Colombia with 4,508 cases notified in 2021. Public health reference laboratories play an essential role in epidemiological surveillance of the disease and CL-case confirmation by direct microscopic examination (DME) of lesion scrapings and supervise the quality control of the tests applied in other laboratories by the external performance evaluation programs. DME has a high specificity (>98%) but a variable sensitivity (54% - 94%) since it relies on variables such as sample quality, parasitic load in the lesion, the expertise of the professional who performs the test, among others. In addition, DME is not able to differentiate *Leishmania* species, which is decisive to guide the patient's treatment accurately and it is associated with the evolution of the disease. In order to overcome these drawbacks, molecular approaches have been exploited such as PCR targeting the minicircles in the kinetoplast DNA (kDNA PCR), a region with approximately 10,000 to 20,000 copies, which makes it a highly sensitive and specific marker for parasite detection. Considering the above, this study evaluated the performance of DME carried out in a public health reference laboratory against kDNA PCR plus identified the *Leishmania* species

infecting patients from Colombia. To evaluate the performance of DME carried out at "Laboratorio de salud pública de la secretaría distrital de salud de Bogotá" (LSP-SDS) against kDNA PCR, which was previously validated, 100 direct smear samples were initially evaluated by microscopy. DNA was later obtained to perform the molecular test as reference standard and the operating characteristics were determined using Stata v.13. Additionally, typing of positive samples was performed by PCR-RFLP (*miniexon* and *hsp70*) and sequencing (*cytb*) approaches. The analysis showed DME has a sensitivity of 88% (95% confidence interval (CI); 75.7% - 95.5%) a specificity of 96% (95%CI; 86.3% - 99.5%) and an accuracy of 92% (95%CI; 84.8% - 96.5%) with a positive likelihood ratio of 22 (95%CI; 5.64 - 85.9) and a negative likelihood ratio of 0.12 (95%CI; 0.06 - 0.27). Furthermore, the species identified corresponded to *Leishmania panamensis* (n=20), *Leishmania braziliensis* (n=12) and the *Leishmania guyanensis / L. panamensis* group (n=11). It was not possible to typify 7 samples. In conclusion, our results indicate the DME carried out by LSP-SDS has a "good" performance at parasite detection. Nevertheless, the application of molecular methodologies like kDNA PCR, with better-operating characteristics; PCR-RFLP, and sequencing is recommended to improve the epidemiological surveillance of the disease.

Keywords CUTANEOUS LEISHMANIASIS; SENSITIVITY; SPECIFICITY; PUBLIC HEALTH SURVEILLANCE; MOLECULAR TYPING

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P1-022: ASSOCIATION BETWEEN HISTOPATHOLOGICAL FINDINGS AND THE DIAGNOSIS OF CUTANEOUS LEISHMANIASIS, CONFIRMED BY PCR, IN AN ENDEMIC REGION OF BRAZIL

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The diagnosis of cutaneous leishmaniasis (CL) is a difficult task and the correct use of histopathological criteria can be useful in clinical practice. The present cross-sectional study evaluated the association of histopathological criteria with the results of polymerase chain reaction (PCR) of clinically suspected cases of cutaneous leishmaniasis (CL). Skin samples were received during 9 years of clinically suspected cases of CL in a Brazilian municipality. From the 222 samples, 190 (85,6%) were positive in PCR. All 25 cased identified in histopathological examination were also positive in PCR. With the exception of more intense inflammatory infiltrate, all other evaluated histological variables (ulceration, epidermal hyperplasia, hyperkeratosis, presence of granuloma, neutrophils, histiocytes, lymphocytes, plasmocytes and necrosis) did not have statistical significant associations with the result of the PCR. The data from the present study shows that the presence of intense inflammatory infiltrate is highly suggestive of the occurrence of CL. On the other hand, the other histopathological findings in isolation cannot be good predictor of PCR positive results.

Keywords CUTANEOUS LEISHMANIASIS; DIAGNOSIS; PCR; HISTOPATHOLOGY

P1-024: MULTIPLEX-PCR FOR THE DETECTION OF *Leishmania* IN A MAMMALS SAMPLE

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Leishmaniasis is considered a global health problem due to its severity, expansion and incidence. The disease is caused by protozoa of the genus *Leishmania*, which are transmitted by the bite of female dipterans of the subfamily Phlebotominae. The dog is the main reservoir host of the parasite in urban areas. In humans, the disease can progress to death in more than 90% of untreated cases. Thus, the importance of developing diagnostic methods for better and faster identification of *Leishmania* and promoting its epidemiological control. This project aimed to standardize the Multiplex-PCR technique for detection of *Leishmania* DNA and mammals DNA in one reaction. Initially, peripheral blood samples from dogs with 10^4 promastigotes of *Leishmania (Leishmania) amazonensis*, *Leishmania (Viannia) braziliensis* and *Leishmania (Leishmania) infantum* were used. DNA was obtained using the 20% SDS technique, according to the previously established protocol. The DNA obtained was resuspended in TE buffer (10mM TRIS; 1mM EDTA; pH 8.0). After obtaining the DNA, the samples were submitted to PCR using two pairs of primers that amplify specific regions of *Leishmania* (13A/13B) and the mammalian β-actin gene (ActinF/ActinV). Annealing temperatures, number of cycles, analytical sensitivity were analyzed and its applicability in different mammals species (dog, cat and human) was tested. The best annealing temperature (55.4°C), the number of cycles (35) and the analytical sensitivity (0.1 fg/µL for *L. amazonensis*, *L. braziliensis* and *L. infantum*) were defined. After standardization, multiplex-PCR was able to detect dog, cat and human DNA, as well as different *Leishmania* species. Under the established conditions, it

was possible to simultaneously amplify the DNA of different *Leishmania* species as well as the DNA of mammals, with great sensitivity. The following work is more advantageous to the traditional PCR method due to its greater agility, speed and less use of reagents.

Keywords DIAGNOSIS; MULTIPLEX-PCR; *Leishmania*; MAMMALS

P1-025: AN OVERVIEW ON LABORATORY APPROACH OF AMERICAN CUTANEOUS LEISHMANIASIS IN THE LAST TWENTY-TWO YEARS AT EVANDRO CHAGAS INSTITUTE, PARÁ STATE, BRAZIL

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American cutaneous leishmaniasis (ACL) represents a serious public health problem in Brazil in reason of its high incidence and wide distribution, but also because of the risk of evolving into severe forms in the absence of laboratory diagnosis and treatment. Laboratory diagnosis is of great importance in the prognosis and treatment as well as in disease control actions. The Leishmaniasis Laboratory "Ralph Lainson" at Evandro Chagas Institute, Pará, Brazil, has been carrying out ACL diagnosis and surveillance for more than five decades, mainly in the Brazilian Amazon, which motivated a descriptive/retrospective population-based study, using secondary data from records of confirmed cases in that laboratory, in the last twenty-two years (1996-2018). The diagnosis was based on epidemiological and clinical data, and laboratory tests [Montenegro skin test (MST), parasite search, isolation, and identification of *Leishmania* sp. by monoclonal antibodies, isoenzyme electrophoresis, and molecular biology-PCR]. A total of 3,687 patients were examined, of which 2,131 had diagnostic confirmation of the disease. Parasite search was performed in 1,734 patients, being positive in 1,302 (75.1%), while MST in 1,716, with a positive result in 1,633 (95.2%). Among the cases with a positive parasite search (1,302), MST was performed in 915 with a positive result in 806 (88.1%). Of the 432 patients with negative parasite search, MST was positive in 100%, evidencing the importance of this diagnostic tool in ACL. In 2001 and 2014 years, a greater number of cases were diagnosed (128 and 124), and in 2016 the lowest number (60). ACL had the highest number of male subjects

(1,746-81.9%), with the highest number between 20 and 59 years old (75.9%). The localized clinical form (LCL) was the most prevalent 1906 (89.4%), with a single lesion in 64.2% of the cases. The mucosal form (LM) was recorded in 229 (10.7%) cases, of which 126 (55.0%) had only nasal lesions. The chronic anergic diffuse form (ADCL) recorded only 8 (0.37%) cases. In terms of the original site of the disease, it was observed that 1,943 cases originated in sixteen different states of Brazil, with emphasis on Pará (87.1%), Maranhão (5.5%), Amapá (3.4%), and Amazonas (1.6%). Of the 144 municipalities in Pará, there were records in 123 (85.4%), especially Paragominas (11.1%), Thailand (5.2%), and Tomé-Açú (4.7%). Cases imported from neighboring countries were 175, mainly from French Guiana (82.8%) and Suriname (13.2%). A total of 770 *Leishmania* sp. samples were isolated, the majority (96.4%) of the subgenus *L. (Viannia)*, with a predominance of *L. (V.) braziliensis* (35.4%), *L. (V.) shawi* (24.8%) and *L. (V.) guyanensis* (15.6%), while the minority (3.6%) of the subgenus *L. (Leishmania)*, 100% *L. (L.) amazonensis*. These results provided a better understanding on the diagnostic situation of ACL in the area covered by the Evandro Chagas Institute, especially in the Brazilian Amazon and neighboring countries.

Keywords AMERICAN CUTANEOUS LEISHMANIASIS; LABORATORY APPROACH; EVANDRO CHAGAS INSTITUTE; BRAZILIAN AMAZON

Financing Evandro Chagas Institute/Surveillance Secretary of Health, Ministry of Health, Brazil.

P1-027: EVALUATION OF THE ACCURACY OF REAL-TIME PCR-HSP20 FOR DETECTION OF *Leishmania* spp. IN TISSUE SAMPLES OBTAINED FROM CONFIRMED LEISHMANIASIS PATIENTS

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Leishmaniasis is a major problem for public health, especially in tropical and subtropical countries, due to the magnitude of the risk population, the physical and emotional consequences and limited effort in governmental to control and eradication. In America, it is caused for more than 20 *Leishmania* species, being *Leishmania infantum, Leishmania braziliensis* and *Leishmania guyanensis* with major incidence. The confirmatory diagnosis is developed means Direct Microscopic Examination for Giemsa staining smears (DME), *in vitro* culture, microculture or histopathological examination of the skin biopsy specimen, methods that reach a sensibility of 85%, 40%, 94-100% and 60%, respectively. In this sense, our research is oriented toward the search for target gene that allow the accurate detection of all Leishmania species. The standardization and evaluate the real-time PCR-Hsp20 with SybrGreen was done primers 5′-GCCRGARGTGARRAAGGAGGAC-3′ and 5′-GYAGCTGGYKYTCGTCCTGC-3′, reported to Montalvo *et al.,* (2020), that recognize the Hsp20 gene of all *Leishmania* species. The analytic sensibility and specificity were evaluated with DNA samples from *Leishmania braziliensis* MHOM/BR/75/M2904; *Leishmania guyanensis* MHOM/BR/1975/M1176; *Leishmania panamensis* MHOM/PA/71/LS94; *Leishmania peruviana* MHOM/PE/LCA08; *Leishmania lainsoni*

MHOM/PE/88/BAB1730; *Leishmania amazonensis* MHOM/BR/1972/M2269, *Leishmania mexicana* MNYC/BZ/62/M379; *Leishmania tropica* MHOM/SU/74/K27, *Leishmania aethiopica* MHOM/ET/72/L100, *Leishmania major* MHOM/SU/73/5-ASKH, *Leishmania infantum* MHOM/TN/80/IPT1 and *Leishmania chagasi,* and other phylogenetically close pathogens as *Trypanosoma cruzi, Chritidia* spp and other distant such as *Plasmodium vivax* and *Plasmodium falciparum,* respectively. The diagnostic sensibility was obtained from 257 DNA samples parasitology confirmed DME or Culture; this group was conformed 129 and 128 from tissue samples from stainless steel lancets and smears Giemsa-staining, respectively. While diagnostic specific was determined in 66 tissue samples from ulcerative lesions of patients with Montenegro skin test negative. All samples included in this study were obtained in specialized diagnostic frame of disease between 2013 and 2021. As a result of the standardization of real-time PCR, the efficiency related to the amplification of nucleotide acids were >90%. While analytic sensibility and specificity both were 100%. The diagnostic sensibility and specificity (Ct≤35) were 89.7% and 100% respectively. Independently, the sensibility in DNA samples from stainless steel lancets were 91.8%, in contrast, from in DNA samples from DME were 87.8%. Montalvo et al., (2020), in a conventional PCR-Hsp20, reported a sensibility and specificity of 94.44% and 100% with predictive positive values and negative of 100% and 88.1% respectively. In this study, we found similar values of 94.3% of sensibility in DNA samples from the stainless steel lancets. In DNA samples from DME, the sensibility found were 91.3%. This difference probably was due to conservation form of Giemsa stained smears and exposition to agents such as immersion oil and Giemsa dye, while the samples obtained from stainless steel lancets were preserved embedded in alcohol at 96° and stored at -20°C. The real-time PCR-Hsp20 standardized and evaluated reached high sensibility and specificity that allowed the accurate detection of different *Leishmania* species and could be applicable to molecular diagnostic and Leishmaniasis incidence studies.

Keywords *Leishmania;* Leishmaniasis; HSP20 HEAT-SHOCK PROTEINS; NEGLECTED DISEASES

P1-028: DEVELOPMENT OF A LATERAL FLOW ASSAY STRIP USING ANTIGENIC OLIGOPEPTIDES opH2A AND opLiP2a FOR SERODIAGNOSIS OF CUTANEOUS LEISHMANIASIS

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In Peru, cutaneous leishmaniasis (CL) is a public health problem that predominantly affects the socio-economically depressed rural population. Considering that most patients with the disease live in areas where primary care services are precarious or non-existent, both in terms of human and technological resources, there is a need for a simple, fast, and accurate test for the diagnosis of these patients. Two oligopeptides opH2A and opLiP2a, internationally patented by our laboratory (WIPO/PCT: WO2015001383) showed a sensitivity and specificity of 82.04% and 98.67% respectively by the ELISA method, despite its good performance, this technology is not applicable to the small health services that are the closest to leishmaniasis patients. Methods based on lateral flow assay (LFA) have many advantages such as, they are easy to perform and interpret, are low cost, and they don't require sophisticated laboratory instruments or highly trained personnel. All this makes LFA the best alternative for serodiagnosis of CL. However, LFA presents significant challenges in finding the optimal oligopeptide binding conditions that ensure optimal diagnostic sensitivity and specificity. For this, different solid matrices (FF080HP, FF120HP y FF170HP de Whatman/GE), two approaches of binding biotinylated oligopeptides to anchor proteins (streptavidin and antibiotin) that bind to the nitrocellulose membrane, pHs, temperatures, and blocking agents. The visible signal was tested with HRP-DAB, colloidal gold nanoparticles and gold nanoshells. The optimal conditions were obtained using the FF120HP nitrocellulose membrane with both oligopeptides and it was selected the antibiotin as

anchor protein with a concentration used of 500 µg/mL for 100 µg/mL of opLiP2A; in the case of opH2A, 25 µg/ml were used with streptavidin at 200 µg/mL concentration; this last oligopeptide obtained a detection level comparable to the ELISA format. These conditions together applied to the LFA strip, provided a result at 40 minutes in both oligopeptides, likewise this assay presented a better sensitivity through detection with gold nanoshells and was able to discriminate between the presence and absence of the visible reaction of the positive and negative serum controls for leishmaniasis, respectively. The LFA strips developed in this work, are currently being evaluated as a diagnostic tool for a panel of sera samples from leishmaniasis-positive patients and healthy volunteers, and the results will be presented in the poster.

Keywords CUTANEOUS LEISHMANIASIS; SERODIAGNOSIS; opH2A; opLiP2a; LATERAL FLOW ASSAY

Support CONCYTEC - The World Bank (095-2018-FONDECYT-BM-IADT-AV)

P1-029: DETECTION OF *Leishmania* PARASITES AND L*eishmania* RNA VIRUS 1 IN CUTANEOUS LESIONS AND HEALTHY NASAL MUCOSA OF PATIENTS FROM BRAZILIAN AMAZON

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The evolutionary course of tegumentary leishmaniasis (TL) infection is still not clarified. It is known that a percentage of patients may have hematogenous dissemination of parasites with potential involvement of the nasal and/or oral mucosa (mucosal leishmaniasis - ML). One of the factors associated with a greater chance of developing ML is the presence of Leishmania RNA Virus 1 (LRV1), a potent innate immunogen that contributes to the survival of Leishmania, resulting in parasite persistence and dissemination. The state of Rondônia, located in the Western Amazon, has a wide diversity of vectors and Leishmania species and has high rates of ML notification. In addition, about 30% of Leishmania (Viannia) parasites in this region have the viral endosymbiont LRV1. Some studies point out that the early detection of parasites in the healthy nasal mucosa may represent a poor clinical prognosis and a sign of dissemination. Since the diagnosis of ML at an early stage is uncommon, the correlation between the presence of parasites in healthy mucosa opens an opportunity for new clinical approaches to TL care. Thus, we aimed to investigate the relationship

between the presence of parasites in the healthy nasal mucosa and the detection of LRV1 in patients with Localized CL (LCL) before (day 0, D0) and after (day 20, D20) treatment with pentavalent antimonial. The evolution of the patients has been followed in the period of 90 to 180 days after its inclusion in the study. The diagnosis of LCL was confirmed by direct parasitological examination and qPCR. The detection of LRV1 and the quantification of the parasite load were performed by qPCR of samples obtained by collection with a cervical brush. Samples from 30 patients with LCL were analyzed. Of these, 19 patients completed the entire proposed 180-day clinical follow-up. Samples from eight patients (42.1%) were positive for LRV1 in the lesion and samples from six patients (31.5%) were positive in qPCR of healthy nasal mucosa. Six patients (31.5%) had both positive variables, LRV1 in the lesion and qPCR Leishmania in the healthy mucosa. Eleven (57.8%) patients still had an unhealed skin lesion after treatment (D20). Among those, one (9.09%) showed infiltration in the lesion, despite being completely re-epithelialized, evolving with ulcer recurrence and the visualization of parasites by direct examination and detection by molecular tests on D90. On D20, among the 11 unhealed, parasites were detected in the molecular analyzes in 3 (27.2%) patients. At the end of the D90 evaluation, 4 patients had unhealed CL, 3 of them with detection of Leishmania in the lesion. Of these 4 patients, two was LRV1 positive on D0. As ML is silent until it reaches advanced stages, understanding the dynamics and distribution of Leishmania in the human host is essential for directing control measures and their assessment. The detection and quantification of parasites is important to determine infection status, monitor treatment and address gaps in understanding the natural history of human infection. These results may contribute also to the reflection on the adoption of therapeutic regimens.

Keywords CUTANEOUS LEISHMANIASIS; PARASITE DISSEMINATION; LEISHMANIA RNA VIRUS; LRV1; CLINICAL FOLLOW-UP

Financing INCT-EpiAmo; Programa de Pesquisa para SUS MS-DECIT/FAPERO; Programa de Excelência em Pesquisa da Fiocruz RO

P1-030: *Leishmania sp*. IN SUBLINGUAL NODLE OF BITCH DIAGNOSED THROUGH CAPILLARY CYTOLOGY

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Leishmania sp. is an intracellular protozoan of the Trypanosomatidae family that has two most relevant evolutionary forms: amastigote and promastigote. *Leishmania (L.) infantum* amastigotes parasitize cells of the mononuclear phagocytic system of vertebrate hosts, and can infect the most diverse tissues, both in humans and animals. The objective of this work was to report the presence of *Leishmania* sp. in sublingual nodule of bitch diagnosed by capillary cytology. In March 2021, in a veterinary clinic in the city of Parnaíba, Piauí, Brazil, the animal was diagnosed with Visceral Leishmaniasis (VL), first by the cytology technique in a sublingual lesion and later by medullary puncture. These exams confirmed the presence of *Leishmania* sp. The Golden Retriever bitch, just over 1 year old, weighing 30 kg, was vaccinated with three doses of Leish-Tec® and used a repellent collar with deltamethrin as an active principle. The tutor sought veterinary care because the animal had been presenting fever and difficulty swallowing for a few days. Physical examination revealed a hyperemic sublingual nodule with pus and a foul odor in the mouth. Marbofloxacin 2.75 mg/kg and prednisolone 1 mg/kg were prescribed daily for 5 days before the VL diagnosis was confirmed. The hemogram observed normocytic hypochromic anemia, thrombocytopenia, eosinopenia, lymphopenia and absolute monocytopenia, and serum biochemistry revealed an increase in alkaline phosphatase and inversion of the albumin/globulin ratio, in

addition to hepatosplenomegaly, alterations suggestive of VL. After the diagnosis of the disease, therapy with Miltefosine at a dose of 2mg/kg/day for 28 days and allopurinol 10mg/kg every 12 hours for 1 year was recommended, but during this period the nodule disappeared and months then it would come back, compromising the animal's health, until the owner authorized euthanasia. VL is a serious chronic disease that may present nonspecific clinical signs confused with other diseases. The parasitized animal can remain asymptomatic for months and progress to the most diverse clinical manifestations, however lesions in the oral cavity are rarely reported. The presence of the protozoan in the sublingual nodule in the bitch in question can be explained by the recruitment of inflammatory cells parasitized with *Leishmania* sp., resulting in an exacerbated inflammatory process, in addition to opportunistic infections, which may have occurred in this animal. It is concluded that in veterinary medicine a complete physical examination of dogs is important, since a good clinical evaluation and an early diagnosis can identify atypical lesions of VL and prevent further damage to the health of animals, especially in endemic areas.

Keywords AMASTIGOTE; DOG; CYTOLOGY; VISCERAL LEISHMANIASIS

P1-031: DEVELOPMENT OF A TAQMAN REAL TIME PCR ASSAY BASED ON MINI-EXON GENE TO DETECT *Leishmania Viannia* SPECIES AND DETERMINE PARASITE LOAD IN CLINICAL SAMPLES

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American Cutaneous Leishmaniasis (ACL) is a neotropical disease caused by parasitic protozoa from genera *Leishmania* and transmitted by sand fly vectors. It is considered a neglected disease globally afflicting 2 million people and 350 million people live at risk of infection. In Panama, Leishmaniasis is considered a public health problem that presents an estimated annual incidence rate between 1000 to 3000 new cases. Real Time PCR approaches based on kDNA and rDNA markers are the most widely used for both detecting parasites of *Viannia* subgenus and determining their load in clinical and biological samples. However, despite of the high sensibility of these markers, some specificity problems have been already pointed out for both molecular targets. This fact complicates the accurately detection of *Leishmania* parasites as well as the determination of their load in clinical samples from regions where this parasite overlaps in distribution with other trypanosomatids. Consequently, it is necessary to evaluate more specifics targets that permit a reliable detection of *Leishmania* parasites at genus or subgenus level. In this sense, we developed and evaluated a Real time PCR assay using the mini-exon gene as molecular marker. Two-hundred partial and complete mini-exon gene sequences from *Leishmania* species and other trypanosomatids were retrieved from GenBank and aligned using the MAFFT algorithm within the UGENE bioinformatic platform. We used the primer 3 algorithm contained in the same platform to design ten sets of primers and probes that were further

evaluated using primerBLAST and OligoAnalyzer software to choose the more specific set that produce no secondary structures. Selected primers and probe were then used to develop a qPCR specific for *Viannia* species that showed a dynamic extension ranging from 10^5 to 10^{-1} parasites equivalents/reaction and no amplification of *Trypanosma cruzi*, and *Trypanosoma rangeli* DNA. After finding the best qPCR conditions using genomic DNA from reference strains of *Viannia* subgenus, we compared our qPCR with a conventional assay targeting a kDNA minicircle specific for this subgenus. Our preliminary results with 50 clinical samples indicated a 100% of concordance between both techniques. We also were able to multiplex our qPCR approach with RNAse P human target without minimizing the amplification limit of this technique (10^{-1} parasites equivalent/ mL). Analyses are undergoing to correlate the normalized parasite load obtained by this technique and the one obtained by a qPCR assay using kDNA as a specific target for *Leishmania Viannia* parasites.

Key words *Leishmania Viannia*, PANAMA, PARASITE LOAD, MINI-EXON, KDNA, QPCR

P1-032: *Leishmania* DNA DETECTION IN HUMAN SAMPLES USING CRISPR-CAS12A

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Tegumentary leishmaniasis manifests as skin ulcers and/or severe disfiguring mucosal lesions that affect poverty-related populations and have a significant public health impact in the Americas. The disease is caused by different *Leishmania* species, in most cases due to species belonging to the *L*. (*Viannia*) subgenus, with predominance of *L*. (*V*.) *braziliensis*. Molecular diagnostics are the most sensitive and specific techniques currently used for both detecting and identifying the infecting parasite species. Unfortunately, these techniques require highly specialized infrastructure and trained personnel that are not available in low-resource primary care settings, i.e. in rural endemic areas. CRISPR-Cas systems, naturally occurring in bacteria and archaea, have been repurposed for genome editing and more recently for nucleic acid detection. CRISPR-Cas nucleases offer the advantage of being programmable by the corresponding CRISPR RNA (crRNA) of choice, unlocking any target nucleic acid recognition with single-nucleotide specificity. CRISPR-based detection is enabling the development of *in vitro* diagnostic solutions at the point-of-care. Here, we harnessed the CRISPR-Cas12a system to develop assays capable of detecting different *Leishmania* species of medical importance. Our assays employ multi-copy targets that are widely used in the molecular diagnosis of leishmaniasis: the highly conserved 18S ribosomal RNA gene (18S rDNA) for pan-*Leishmania*

detection and a kinetoplast DNA (kDNA) minicircle region conserved among *L*. (*Viannia*) species. Cas12a crRNAs were carefully selected *in silico* for *Leishmania* target sequences, thereby filtering against the human genome and genomes from related pathogens that co-circulate in leishmaniasis endemic regions and/or cause leishmaniasis-like lesions. Our workflow combines crRNA-guided Cas12a detection coupled to PCR preamplification to ensure high detection sensitivity. The analytical validation using laboratory reference strains showed high detection sensitivity, down to 5×10^{-2} (kDNA) vs. 5×10^{0} (18S rDNA) parasite genome equivalents per reaction. The assays were specific for the tested *Leishmania* species, while no cross-reaction was observed with *Trypanosoma cruzi* or human DNA. We then applied our optimized workflow on a panel of 49 patient's skin lesion samples. Based on the results achieved, both novel CRISPR-based assays showed robust performance, with high positive (>80%) and negative (100%) percent agreement compared to a kDNA-based quantitative real-time PCR assay. Our assays hold potential as alternative to real-time PCR for use in reference and research laboratories. In addition, the assays can be integrated into low-cost, portable PCR and fluorimeter devices to be applied in rural settings. Both assays are also amenable to further optimization and simplification, including introducing isothermal preamplification and point-of-care readout, to facilitate their use in the field. This opens the way to a new generation of versatile and accurate molecular tools with diverse potential applications in leishmaniasis diagnosis, surveillance and research, including One Health approaches to control the disease.

Keywords TEGUMENTARY LEISHMANIASIS; *Leishmania*; CRISPR-CAS; NUCLEIC ACID DETECTION; MULTI-COPY TARGETS

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P1-033: SARS-COV-2 SPIKE RECEPTOR-BINDING DOMAIN (RBD) EXPRESSED BY THE PARASITE *Leishmania tarentolae*: APPLICATION IN THE SERODIAGNOSIS OF COVID-19

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After the isolation and description of SARS-CoV-2, serological tests for COVID-19 diagnosis were developed mainly based on the use of antigens produced in human cells. However, there are potential limitations related to the use of viral antigens produced by mammalian cells, particularly for rapid application and fast production in developing countries. Recently, the protozoan parasite *Leishmania tarentolae* has been suggested as a new eukaryotic system for the expression of recombinant proteins, which stands out for its easy handling, non-pathogenicity, and the capability to produce antigens with glycosylation pattern similar to that of vertebrates. Here, we explored the use of *L. tarentolae* for the expression of the receptor-binding domain (RBD) of the SARS-CoV-2 virus applied for the serodiagnosis of COVID-19. The parasite *L. tarentolae* was engineered for the extracellular

expression of RBD protein, then the purified antigen was tested to detect specific anti-SARS-CoV-2 antibodies in human sera, setting-up and validating an in-house ELISA assay according to the standard criteria of specificity, precision, accuracy, and robustness. Then, a comparative analysis to assess the validity of the in-house ELISA assay was carried out on a cohort of 80 human sera collected from asymptomatic subjects during the pandemic period of March/April 2020. This experimental work showed that the protein RBD produced using the *L. tarentolae* system possess folding and glycosylation patterns comparable to that of the same antigen produced in mammalian cells. In addition, the qualification process of the serological test for the detection of specific anti-SARS-CoV-2 antibodies, set-up using the antigen produced in *L. tarentolae*, fulfilled all the acceptability criteria defined in the validation assay. Finally, the analysis on 80 human sera revealed the ability of the recombinant RBD to detect specific antibodies, comparable to that guaranteed by RBD produced in mammalian cells, obtaining an almost perfect match with just one mismatch. In conclusion, we provide the first evidence on the potential utility of *L. tarentolae* as a microfactory to produce antigens for serodiagnosis of Coronaviridae infections, and as an effective system for viral antigen production, even in countries that lack high-technology cell factories.

Keywords SERODIAGNOSTICS; *Leishmania tarentolae*; RECOMBINANT PROTEIN; SARS-COV-2

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P1-034: IT-LEISH: A VITAL TEST IN THE ELIMINATION OF VISCERAL LEISHMANIASIS

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The IT-Leish, widely used for the diagnosis of visceral leishmaniasis (VL), is the only rapid diagnostic test (RDT) with high enough performance in East Africa and is used by Médecins Sans Frontières (MSF). Bio-Rad Laboratories, the current manufacturers of the test, are to discontinue the manufacture of the IT-Leish in 2022. As of May 2022, the In Vitro Diagnostic Regulation (IVDR) comes into effect within the EU, presenting a major change to the regulatory framework for in vitro diagnostic medical devices including RDTs. CE marking under the IVDR requires a higher financial cost in comparison to CE marking under the In Vitro Diagnostic Directive. For tests with small markets, compliance with the IVDR may not be cost effective. Without continued manufacture of the IT-Leish, the World Health Organization's (WHO) 2021-2030 road map for neglected tropical diseases in alignment with the Sustainable Development Goals may be under threat. Mologic is a company limited by guarantee and as such has the capacity to place tests manufactured at low volume and supplied at low cost on the market. Mologic are currently liaising with Bio-Rad Laboratories for technology transfer of IT-Leish.

Keywords VISCERAL LEISHMANIASIS; IT-LEISH; rK39; DIAGNOSTIC

P1-035: EVALUATION OF AN rK28 BASED RAPID DIAGNOSTIC TEST AGAINST TISSUE ASPIRATE MICROSCOPY FOR VISCERAL LEISHMANIASIS DIAGNOSIS, ETHIOPIA 2019-2021

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Ethiopia is a high burden country for visceral leishmaniasis and, as in other countries in eastern African the available rapid diagnostic tests (RDT), which use the recombinant antigen rK39, have low sensitivity. This is why one of the priorities highlighted in the WHO Road Map for NTDs, 2021-2030 is the need for, 'more sensitive rapid diagnostic tests for use in East Africa'. Prototype RDTs based on the rK28 recombinant fusion antigen showed high sensitivity in Ethiopia, but their performance in large prospective studies against the VL diagnostic algorithm, including rK39 RDT, had not been assessed yet. Within the frame of the project AfriKADIA, we conducted a prospective evaluation of an rK28 RDT (CTK Biotech) with consecutive recruitment of VL suspected cases attending the Leishmaniasis Research and Treatment Centre - University of Gondar, Gondar, Ethiopia between 2019-2021. Participants were classified as VL case / non-VL case based on diagnostic protocol of the LRTC, which includes rK39 RDT and spleen aspirate microscopy, but not necessarily confirms VL based solely on a positive serology if other causes of disease are confirmed. Using a sub-sample of those recruited we nested a study to evaluate another RDT detecting IgG1 against whole *Leishmania* antigen (VL Sero-K-Set, CORIS

BioConcept). Three hundred and eighty-nine VL suspects were recruited, 99 (25.5%) VL cases and 290 (74.5%) non-VL cases. The rK28 RDT returned a 98.99% [95%CI 96.5-100] sensitivity and 39.6% [95%CI 33.8-45.4] specificity for blood samples and 100% [95%CI 99.5-100] sensitivity and 32.7% [95%CI 27.28-38.33] specificity for serum. The IgG1 RDT showed 98.4% specificity [95%CI 96.5-100] in blood and 86.5% in serum samples [95%CI 82.4-90.6]. The low specificity of the rK28 RDT can be explained by cross-reactivity with malaria, as shown in other recent study in the same setting, as well as with other diseases. And this does not support including this rK28 RDT as first line test in the VL diagnostic algorithm. Specific detection of IgG1 against rK39 and rK28 antigens can be explored for a more sensitive test for monitoring treatment outcome.

Keywords VISCERAL LEISHMANIASIS; RAPID DIAGNOSTIC TESTS; ETHIOPIA; EASTERN AFRICA

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P1-036: AGREEMENT STUDY OF TWO ANTIBODY DETECTION DIAGNOSTIC TESTS FOR VISCERAL LEISHMANIASIS: rK28-BASED RAPID DIAGNOSTIC TEST AND DIRECT AGGLUTINATION TEST

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According to the visceral leishmaniasis (VL) diagnostic algorithm in most national guidelines in eastern Africa, the direct agglutination test (DAT) is the second serological test applied, after rK39-based rapid diagnostic test (RDT). A positive DAT result can trigger treatment. And suspected VL cases that are DAT-borderline or DAT-negative with high suspicion of being a VL case are referred for tissue aspirate microscopy. This approach assumes high sensitivity and specificity for DAT. Therefore, tests intended to replace the rK39-DAT combination in the VL diagnostic algorithm must have a good agreement with DAT. There is a pressing need to identify RDTs with high sensitivity that can be included in the VL diagnostic algorithm. Studies with RDTs based on the chimeric antigen rK28 have shown these can be good candidates. Within the frame of the project AfriKADIA, we conducted a retrospective analysis of frozen plasma samples from VL suspects and cases. We studied a panel of 246 samples from VL foci in South Sudan and Ethiopia: 95 samples from newly diagnosed VL episodes, 62 samples form VL patients on treatment, 87 samples from non-confirmed VL suspects, 2 fever cases non-VL. We tested in parallel with this panel a DAT test produced at the University of Amsterdam Medical Centre (same as the formerly produced at the Royal Tropical Institute, KIT) and an rK28 RDT produced by CTK Biotech. The agreement between the rK28 RDT and DAT was found to be moderate (observed agreement 0.86, expected agreement 0.52, Kappa 0.71

with a 95% Confidence Interval of 0.61-0.80. This is not an encouraging result as strong agreements (>0.80 –0.90) or almost perfect agreements (>0.90) would be more acceptable if there is an intention of replacing DAT by rK28 RDT.

Keywords VISCERAL LEISHMANIASIS; RAPID DIAGNOSTIC TESTS; EASTERN AFRICA

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P1-037: ASSESSMENT OF CROSS-REACTIVITY WITH MALARIA OF TWO ANTIBODY DETECTION DIAGNOSTIC TESTS FOR VISCERAL LEISHMANIASIS: rK28 RDT (CTK Biotech) and DAT (AMC)

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Preliminary studies have shown that rapid diagnostic tests (RDTs) based on the chimeric antigen rK28 have high sensitivity for diagnosing visceral leishmaniasis (VL). But cross-reactivity of rK28 RDTs, either the test produced by CTK Biotech or prototypes from other companies, with malaria or other infectious diseases (e.g., tuberculosis, schistosomiasis) has also been a concern. Within the frame of the project AfriKADIA, we conducted a retrospective analysis of 126 frozen serum samples from travellers, migrants, visiting friend and relatives seropositive to infection by *Plasmodium*. Out of this 126 samples, 38 tested positive for *Plasmodium* DNA by PCR. The samples were tested in parallel with a direct agglutination test (DAT) produced at the University of Amsterdam Medical Centre (same as the formerly produced at the Royal Tropical Institute, KIT) and an rK28 RDT produced by CTK Biotech. Out of the 38 malaria seropositive and PCR positive, 7 (18.4%) tested positive for rK28 RDT, while none of them was positive to DAT. For the 88 malaria seropositive and PCR negative, rK28 RDT returned a positive result in 3 (3.4%) of the samples and DAT was negative. We confirm the possibility of rK28 RDTs cross-reacting with malaria. This is important because malaria and VL can be co-endemic, and antibodies to *Plasmodium* are not usually ruled out in the process of VL diagnosis. This

cross-reactivity may be due to the design of the rK28 antigen as there is a 5 amino acid HASPB1 repeats that also occur in *Plasmodium falciparum* (HASPB1 repeats are one of the components of rK28 antigen). These issues could be addressed by redesigning rK28 recombinant antigen to edit those HASPB1 repeats.

Keywords VISCERAL LEISHMANIASIS; RAPID DIAGNOSTIC TESTS; EASTERN AFRICA

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P1-038: APPROACH TO A SYSTEMATIC REVIEW OF THE DIAGNOSTIC PERFORMANCE OF rK28 BASED RAPID DIAGNOSTIC TESTS IN EASTERN AFRICA

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Rapid diagnostic tests (RDTs) using the recombinant antigen rK39 have been extensively evaluated in different endemic regions. These have shown a very good diagnostic performance in Southeast Asia and their uptake has been rapid. Unfortunately, their sensitivity in eastern Africa is suboptimal. RDTs based on the recombinant antigen rK28, may overcome this problem. The rK28 is a synthetic polyprotein containing repeats of rK39, together with those from the K9 (HASPB2) and K26 (HASPB1). Some studies claim that it shows better performance in eastern Africa than the rK39 antigen; however, data are not definitive. Within the frame of the project AfriKADIA, we conducted a systematic review and evidence analysis to put together the available evidence on the diagnostic performance of this test in eastern Africa. We found 153 articles that can be identified in the literature by combining the terms rK28 / K28 AND leishmania, leishmaniasis, visceral leishmaniasis, kala azar, kala-azar, kalazar. After eliminating duplicates only 28 articles remained. Abstracts were reviewed and 21 articles were discarded because they were related either to canine leishmaniasis, leishmaniasis in Southeast Asia or Latin America, they were general reviews or studies specific to genetic polymorphism. Full text review was conducted in 7 articles and another two were excluded because they were not about VL diagnosis in eastern Africa in a defined series of patients. Only 5 articles remained for evidence analysis. We observed a high variability among the studies in these 5 articles in terms of study type (case-control vs consecutive), sample tested (serum, plasma, blood), rK28 RDT used, control groups and reference test, among others. For the studies identified we

focused on 4 key domains, covering patient selection, index test, reference standard, and flow of patients through the study, and applied the QUADAS-2 tool to identify the quality, risk of bias and applicability. This showed that for these 5 studies both the risk of bias and the concerns regarding applicability were high. Also, a pooled analysis of the diagnostic performance of rK28 RDT by type of study (cross sectional or consecutive enrolment), showed that case-control studies tended to overestimate the specificity. Therefore, and due to the limited evidence, recommendations on the use of rK28 RDTs should wait for further evidence through large scale studies with consecutive enrolment.

Keywords VISCERAL LEISHMANIASIS; RAPID DIAGNOSTIC TESTS; rK28; EASTERN AFRICA

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P2-010: MILTEFOSINE FOR THE TREATMENT OF CUTANEOUS LEISHMANIASIS A PILOT STUDY FROM ETHIOPIA

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Cutaneous leishmaniasis (CL) in Ethiopia is caused by *Leishmania aethiopica,* and often severe and hard to treat compared to CL caused by other species. Miltefosine is the only oral anti-leishmanial drug, with a favorable side-effect profile compared to routinely available sodium stibogluconate (SSG). Evidence about its use for *L. aethiopica* is lacking. In an observational cohort study, treatment outcomes, safety and adherence among CL patients who required systemic treatment and received miltefosine for 28 days in Boru Meda Hospital and University of Gondar Hospital were studied. Patient cure was defined as 100% flattening for non-ulcerated lesions and 100% flattening and 100% re-epithelization for ulcerated lesions. Outcomes were documented for day 28, 90 and 180, both per site, and pooled, adjusting for site as a fixed effect with effect coding. Among 94 included patients (32 in Gondar, 62 in Boru Meda), median lesion duration was 12 months, median size six cm, and mucosal involvement (46.8%) and diffuse (30.9%) lesions were common. Adherence to miltefosine was good; gastro-intestinal side effects were common but tolerable. Initial outcomes at day 28 were promising, with 68.8% and 94.0% of patients having good improvement or cure in Gondar and Boru Meda respectively. In Boru Meda, outcomes were good with 72.7% and 72.9% cure at day 90 and day 180 respectively. In Gondar, results were less promising, with only 12.5% and 26.7% cure at day 90 and day 180, although

confidence intervals were wide. In pooled estimates, 48.7% of patients reached cure at day 180, and 32.3% relapsed. Outcomes were better in Boru Meda Hospital, for smaller lesions and for mucosal lesions. The initial good response of miltefosine indicates good antileishmanial activity, but the response does not seem to be maintained in certain patients. We hypothesize that this non-sustained response is due to lack of protective immunity development after drug cessation, needed to combat residual hard-to-reach parasites. Therefore, combining miltefosine treatment with another immunomodulatory drug or extending treatment could potentially improve outcomes in order to achieve stable cure. Based on miltefosine's good initial response, tolerable side-effects, and tablet-form, we propose to include miltefosine for future clinical trials using extended treatment schedules, combination therapy, or targeting specific subgroups.

Keywords *Leishmania aethiopica*; MILTEFOSINE; TREATMENT; RELAPSE; CURE

P2-011: ADVERSE EVENTS DURING MILTEFOSINE TREATMENT FOR CUTANEOUS AND MUCOSAL LEISHMANIASIS

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Cutaneous and mucosal leishmaniasis are endemic diseases in Brazil, marked by limited therapeutic options requiring parenteral administration route until recently. Miltefosine is the first and the only treatment available in oral form for leishmaniasis and it was recently distributed in Brazil. The aim of this study is to present the frequency and the pattern of adverse events (AE) observed in patients treated with miltefosine and recorded by the pharmacovigilance service of a referral center for leishmaniasis in Brazil (Fiocruz Minas). Any new or worsened sign/symptom and laboratory abnormality that arose after the beginning of the miltefosine therapy was considered as AE and registered using the international Medical Dictionary for Regulatory Activities (MedDRA) - a standardized format. The AEs were classified in relation to the affected System Organ Classes (SOC) and the intensity of symptoms based on the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events (mild, moderate, severe, life-threatening, death related to the AE). In turn, seriousness was defined according to the World Health Organization (WHO) criteria. The causal relationship between AEs and miltefosine was assessed according to the WHO-Uppsala Monitoring Center (WHO-UMC). A total of 55 patients treated with miltefosine from December 2018 to February 2022 were analyzed and 41 (74,5%) of them had at least one AE. Among these 41 patients, the leishmaniasis clinical manifestation was cutaneous in 12 patients, mucosal in 21 patients and mucocutaneous in 8 cases. The male: female ratio was 32:9 and the median age was 66 (ranging from 29 to 92) years. One hundred sixty-four AE were recorded, with an average of 5 events per patient. The most common clinical manifestation was related to the

gastrointestinal tract (46%), followed by the musculoskeletal (6%) and the nervous systems (5.5%). Forty out 164 AEs (24%) were laboratorial abnormalities. Serum creatinine (11%) and urea nitrogen (3.7%) increase, elevated liver enzymes (2.4%) and potassium abnormalities (2.4%) were those most reported. The severity of renal impairment was specialty intense in this series, resulting in drug discontinuation in 8 patients. Two clinical serious AE (SAE) were observed in the same patient, an 81 years-old man who developed diarrhea and respiratory symptoms simultaneously, requiring hospitalization. This patient had confirmation of Covid-19 and died within 72 hours of admission to the hospital. The association of both hospitalization and death with miltefosine was classified as unlikely. Due to the small sample size, risk factors for the occurrence of AE could not be identified. This series demonstrates the high frequency of adverse events during miltefosine use, with a predominance of gastrointestinal and renal function alterations. Phase VI studies and pharmacovigilance strategies need to be routinely implemented.

Keywords MUCOSAL LEISHMANIASIS; CUTANEOUS LEISHMANIASIS; MILTEFOSINE; ADVERSE EVENTS; TOXICITY

Financing CAPES, CNPq grant to GC (3013841-2019-3)

P2-012: EFFICACY AND TOXICITY OF DIFFERENT TREATMENTS FOR MUCOSAL LEISHMANIASIS: A SYSTEMATIC REVIEW

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Mucosal involvement is considered one of the most morbid forms of cutaneous leishmaniasis. The treatment of this condition is supported by fragile scientific evidence. We conducted a systematic literature review to evaluate the therapeutic efficacy and toxicity associated to different treatments for mucosal leishmaniasis (ML). The outcomes of interest were clinical cure and adverse events rate reported in any study with more than 10 patients, without language restriction or publication date, restricted to cases originating in the Americas region. PRISMA guidelines for systematic reviews and Cochrane manual were followed. Sources were MEDLINE, LILACS, EMBASE, Web of Science databases and manual search of references from evaluated studies. We included all studies reporting outcomes after ML treatment, regardless of their design. The risk of bias was evaluated by different tools: randomized clinical trials (RCT) by Cochrane risk of bias (RoB 2), non-randomized clinical trials and prospective cohort studies by Newcastle Ottawa Scale (NOS) and retrospective cohort or case-series by modified NOS. Were included 27 studies (7 RCT, 7 non-randomized clinical trials or prospective cohort and 13 observational retrospective studies) totaling 1666 participants with ML. The studies were mainly conducted in Brazil (17), the follow-up period ranged from 1 month to 5 years. Nine studies performed the Leishmania specie identification and *L. brasiliensis* was the most isolated. Pentavalent antimonials (meglumine antimoniate/MA and sodium Stibogluconate/SSG were interventions more studied. Other interventions were pentamidine, miltefosine, imidazoles, aminosidine sulphate, deoxycholate amphotericin B and amphotericin B lipidic formulations (liposomal, lipid complex, colloidal dispersion) . In four studies a combination of pentavalent antimony with another drug was

assessed (pentoxifylline or allopurinol or sulfa). In general, the studies had low methodological quality (at least one domain with a high risk of bias was identified). Comprehensive Meta-Analysis software v.3 was used to perform one-group meta-analysis of study arms with the same drug to estimate global cure rates using an intention-to-treat approach direct comparison was performed when available. The random-effects model was used to combine these studies. The cure rate of patients treated with MA was similar with the usual therapeutic dose (15-20 mg/kg for 10-30 days) or low doses (5mg/kd/day, variable length):.63.5% (CI:51.7-73.9; I^2=60) versus 67.2% (CI:54,3-78,0; I^2=0) respectively.(significant difference was observed in the final cure rate with the lipidic formulations (79.6%, 60.7-90.7%; I^2=63) compared to deoxycholate amphotericin B formulation (39.5%, CI:16.4-68.5; I^2=71), (p=0.0001). Cure rates for the other interventions were: 83.3% (CI: 57.8-94.8%; I^2=42) for pentamidine, 61.7% (*CI:*52.9-69.7%; I^2=0) for miltefosine, 53.2% (CI:23.2-81.1%) for imidazoles and 11.9% (CI:0.0-69.8%; I^2=72) for aminosidine. Except for miltefosine, for all other interventions a high intra-group heterogeneity (wide confidence intervals) could be observed. The only direct comparison possible was between MA and miltefosine, which was carried out by including two RCT: with a universe of 57 treated patients, no difference was observed between these interventions (OR: 1.2, 0.43-3.49, I^2=0). High heterogeneity and limited methodological quality of studies was observed, which requires caution in the interpretation of results. New RCT studies are necessary to compare the different interventions for ML.

Keywords MUCOSAL LEISHMANIASIS; TREATMENT, THERAPY, SISTEMATIC REVIEW *Leishmania (viannia) braziliensis*

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P2-013: NOVEL SINGLE-DOSE INTRALESIONAL TREATMENT OF CUTANEOUS LEISHMANIASIS WITH AMPHOTERICIN B: AMPHO-DEPOT®

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Despite its location in the skin, cutaneous leishmaniasis treatment remains unsatisfactory due to requirement of repeated parenteral injections that cause severe systemic side effects. Local intralesional injections with antimonials also demand repetition due to their high hydrophilicity and blood absorption that can also lead to reported systemic effects. Amphotericin B (AmB) is the most active antileishmanial drug clinically available, but development of an effective topical formulation is challenging due to poor absorption through the skin. In the present work, we describe the development and therapeutic use of a novel AmB formulation based on sustained release delivery system for a single intralesional injection (Ampho-Depot®, mark deposited in 2021). For that, poly(lactide-co-glycolide acid) (PLGA) microparticles loaded with AmB were prepared by the emulsion solvent evaporation method and sterilized by gamma irradiation. AmphoDepot® was then characterized according to sterility, particle size distribution, zeta potential, scanning electron microscopy and encapsulation efficiency. The microparticles exhibited zeta potential of -25.3 mV, spherical shape, and mean diameter of 2.8 ± 0.4 µm (span = 1.55). AmB entrapment efficiency was 73.5 ± 2.6%. No chemical or physical changes were produced by 25 kGy gamma irradiation that effectively prevented contaminated bacteria and yeast growth. *In vivo*, a single i.l. injection into *Leishmania amazonensis*-infected mouse ears revealed that Ampho-Depot® was much more effective in reducing lesion growth than the same dose of

deoxycholate AmB formulation Anforicin®. Measurement of parasite burden on day 56 of infection revealed 86% parasite burden reduction as compared with vehicle controls. Anforicin® reduction was only 32%. These findings indicate that Ampho-Depot® has strong potential as a new safe therapy, and is pharmaceutically ready for Phase IIb clinical trial in patients with CL.

Keywords CUTANEOUS LEISHMANIASIS; AMPHOTERICIN B; POLYMERIC PARTICLES

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P2-014: INTRALESIONAL TREATMENT OF CUTANEOUS LEISHMANIASIS IN PRIMARY HEALTH CARE CENTRES IN TROPICAL AREA: FIRST EXPERIENCE IN BOLIVIA

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The treatment of cutaneous leishmaniasis (CL) in Bolivia is the systemic application of antimoniate of meglumine as first-line as WHO recommended. The purpose was to evaluate the safety and effectiveness of the intralesional application of this drug by health staff of primary health care centers in the tropical area of Cochabamba, Bolivia. Cochabamba notifies more than 300 CL per year, ranking third among nine regions. This is a quasi-experimental study; We aim to recruit 300 subjects with CL in 7 primary health care centers. The health facilities were selected following the criteria: number of CL cases, availability of health staff, and performance of the referral system. The inclusion criteria of subjects were no more than 2 ulcers; diameter of ulcers no more than 900 mm2 and an age of at least 12 years old. All patients signed a written consent to be included in the study, which has the approval of the ethics committee of the Medicine faculty, Universidad Mayor de San Simón. The participation of health care centers in the use of intralesional procedures was gradual. After the training of health staff, five centers during the first year of intervention, two additional during the second year, and

another four during the third year, to complete 10 centers. In the first year, 28 patients were treated intralesionally, and 288 were treated systemically. The second and third years were treated by both procedures 79 and 34 (intralesional) patients and 231 and 288 (systemic); respectively. 156 patients were treated intralesionally; the reason for not reaching 300 (sample size) was outbreaks of social conflicts and the covid-19 pandemic. Of the 156 treated, 154 completed the treatment. In the first-month post-treatment, 70 of them were cured; an additional 22 were cured until the third-month post-treatment, and other additional 14 were cured clinically until six months; of 48 subjets is unknown the clinical outcome post-treatment because they were lost to follow-up. Patient adherence to intralesional treatment was 99% (154/156) and the only undesirable effect was local pain during infiltration. The cure rate reached up to six months post-treatment was 70%. Also was found 6% (9/156) of cases of therapeutic failure. Based on the findings, it is considered that the intralesional application was accepted by both, the staff and the patients because the procedure is simple and the treatment time is half of the systemic treatment but also because there are no serious adverse effects. The percentage of cure clinics achieved is similar to others' previous experience with systemic treatment. Probably, this response to the interaction between parasite and antimony compounds regardless of the drug application procedure. The therapeutic failure of 6% found (6%) is not related to the intralesional procedure because a similar percentage of therapeutic failure was widely reported for systemic treatment by others. The use of intralesional treatment in CL is simple,is accepted by the staff of primary health care centers and also by the patients because there are no adverse effects. Follow-up and monitoring after release from treatment needs to be improved.

Keywords INTRALESIONAL TREATMENT; APPLICATION SCHEMES; CUTANEOUS LEISHMANIASIS

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P2-015: PENTAVALENT ANTIMONY ASSOCIATED WITH G-CSF IN THE TREATMENT OF CUTANEOUS LEISHMANIASIS CAUSED BY *Leishmania (Viannia) braziliensis.*

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Cutaneous leishmaniasis (CL) caused by *Leishmania Viannia braziliensis* in the last decades has decreasing cure rates and long time to heal after treatment with systemic pentavalent antimony (Sb^v) at a high dosage. Granulocyte colony stimulating factor (G-CSF) is associated with epithelialization and healing processes. Randomized controlled double-blind pilot study to compare the efficacy of Sb^v (Glucantime™, 20mg/kg/day intravenously for 20 days) associated with G-CSF and the use of Sb^v (same schedule) plus placebo in the treatment of localized CL. Thirty-two patients aged between 18 and 50 years with localized ulcerated CL (up to 3 lesions) with a PCR positive for *L. braziliensis* were included in the study. G-CSF or placebo (saline solution 0.9%) was applied by intralesional infiltration of 0.1 mL at 4 equidistant points at the edge of the biggest ulcer on day 0 and day 15. Cure was defined as the complete healing without elevation of the ulcer edges on day 90 after initiation of therapy. Failure was defined by the presence of an active ulcer or scar with raised edges at day 90. The 32 patients involved in the study were randomically assigned into two groups: 15 in the control group and 17 in the control group. The sex male predominated in the G-CSF group (59%), while the control group showed a higher frequency of females (53.3%). Illness duration in both groups varied

from 30 to 60 days and the majority of the lesions were localized in the lower limbs. The cure rate was 53% in the G-CSF group and 47% in the control group. G-CSF use in toxic epidermal necrolysis and dystrophic epidermolysis bullosa showed benefit in accelerating epithelialization and healing, besides decreasing the activity of cytotoxic CD8 cells. These actions of G-CSF could have a beneficial effect in the treatment of CL. However, in this pilot trial, we were not able to show a significant higher cure rate in subjects treated with the combination of this cytokine. Parasitic factors involved in therapeutic resistance may be associated with the high failure found in both groups. Our data suggest that the use of G-CSF with standard therapy do not increase the cure rate in CL.

Keywords CUTANEOUS LEISHMANIASIS; TREATEMNT; GRANULOCYTE COLONY STIMULATING FACTOR; PENTAVALENT ANTIMONY

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P2-016: POST-KALA-AZAR DERMAL LEISHMANIASIS (PKDL) DRUG EFFICACY STUDY LANDSCAPE: A SYSTEMATIC REVIEW OF CLINICAL AND OBSERVATIONAL STUDIES TO ASSESS THE FEASIBILITY OF ESTABLISHING AN INDIVIDUAL PARTICIPANT-LEVEL DATA (IPD) PLATFORM

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Post-kala-azar dermal leishmaniasis (PKDL) is a dermatosis which can occur after a conventional treatment for visceral leishmaniasis (VL) and is characterised by macular, papular, nodular, erythematous or polymorphic rashes. Its pathogenesis is unknown but there is cumulative evidence that it is immunologically mediated. PKDL is a public health problem in VL endemic areas, as recent infectivity studies show that *L. donovani* parasites can be found in PKDL lesions, and are a source of infection to sandfly vectors. There are numerous limitations in our knowledge of PKDL, including its pathology, immunology and risk factors. Currently recommended treatments are either expensive (AmBisome), raising safety concerns (antimonials), or are of long durations (miltefosine). We conducted a systematic review to assess the characteristics of PKDL clinical studies, understand the scope of data and to explore the feasibility and value of developing a PKDL individual patient

data (IPD) platform. A review of published literature was conducted to identify PKDL clinical studies by searching the following databases: PubMed, Scopus, Ovid Embase, Web of Science Core Collection, WHO Global Index Medicus, PASCAL, Clinicaltrials.gov, Ovid Global Health, Cochrane Database and CENTRAL, and the WHO International Clinical Trials Registry Platform. Only prospective studies in humans with PKDL diagnosis, treatment and follow-up measurements between 1973 and October 2021 were included. Data was extracted in REDCap capturing variables on patient characteristics, treatment regimens, diagnostic methods, geographical locations, efficacy endpoints, adverse events and statistical methodology. The literature searches identified 3,217 citations, of which 943 unique articles were independently screened by two reviewers. A total of 54 studies (16 clinical trials and 38 prospective observational studies) met the inclusion criteria and were analysed. The studies were conducted in four countries: India (63%; 1983-2021), Bangladesh (11%; 1991-2019), Nepal (4%; 2001-2007) and Sudan (22%; 1992-2021) and enrolled a total of 2,462 patients. Of the 16 clinical trials, 12 were conducted in India (1987-2021), 3 in Sudan (1993-2021) and 1 in Bangladesh (2018) with a total of 21 arms testing 8 different drugs or combinations involving 891 patients. A wide range of heterogeneity in dosage and duration was observed in the different treatment regimens. Antimony formulations and miltefosine each being tested in 33% (7/21) of treatment arms, followed by amphotericin B formulations in 14% (3/21) of arms. Paramomycin alone and in combination with miltefosine was tested in 4.8% (1/21) of arms each. This review provides a landscape of previously and currently tested treatments for PKDL. Only a third of the published studies (and 36% of the patients) were from clinical trials while the other studies were observational. Even in this relatively small number of studies, there is a large variability in treatment regimens tested. Assembling IPD from identified studies can provide granular details on efficacy and safety outcomes and would be a unique resource to answer questions of public health importance that otherwise couldn't be addressed using standalone trials and aggregated published results. This collaborative approach should help generate stronger evidence to be reviewed by policy makers.

Keywords POST-KALA-AZAR DERMAL LEISHMANIASIS; PKDL; SYSTEMATIC REVIEW; EFFICACY; IPD

P2-017: VISCERAL LEISHMANIASIS IN PREGNANCY: A SYSTEMATIC REVIEW

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The occurrence of Visceral Leishmaniasis (VL) in pregnant women is rarely reported in published literature. A systematic review was carried out to identify studies describing VL in pregnancy and summarise the consequences of infection and treatment on the pregnant women and foetus. The following databases were searched: Ovid MEDLINE; Ovid Embase; Cochrane Database of Systematic Reviews; Cochrane Central Register of Controlled Trials; World Health Organization Global Index Medicus: LILACS (Americas); IMSEAR (South-East Asia); IMEMR (Eastern Mediterranean); WPRIM (Western Pacific); ClinicalTrials.gov; and the WHO International Clinical Trials Registry Platform. Since the reports on VL in pregnancy is rare, any clinical reports describing the disease in pregnancy or the cases of vertical transmission of the disease in humans were included in this review. In addition, non-primary research articles such as textbook-chapters, letters, retrospective case descriptions, or reports of accidental inclusion in trials were also considered for inclusion in this review. A total of 272 unique articles were identified from the search, of which 54 records were included in this review. A further 18 records were identified from additional searches of the references of included studies or from personal communication, leading to a total of 72 included records (71 case reports/case series; 1 retrospective cohort study; 1926–2020) included in this review. These

articles described 451 cases of VL in pregnant women. The disease was detected during pregnancy in 398 (88.2%), retrospectively confirmed after giving birth in 52 (11.5%), and the time of identification was not clear in 1 (0.2%). Of the 398 pregnant women whose infection was identified during pregnancy, 346 (86.9%) received a treatment, 3 (0.8%) were untreated, and the treatment status was not clear in the remaining 49 (12.3%). Of 346 pregnant women, Liposomal amphotericin B was administered in 202 (58.4%) and pentavalent antimony in 93 (26.9%). Outcomes were reported in 176 pregnant women treated with Liposomal amphotericin B with 4 (2.3%) reports of maternal deaths, 5 (2.8%) miscarriages, and 2 (1.1%) foetal death/stillbirth. For pentavalent antimony, outcomes were reported in 88 of whom 4 (4.5%) died, 24 (27.3%) had spontaneous abortion, 2 (2.3%) had miscarriages. A total of 26 cases of confirmed, probable or suspected cases of vertical transmission were identified with a median detection time of 6 months (range: 0–18 months). Majority of the published literature on VL in pregnancy were case reports and case series. From the studies identified, it is difficult to derive a generalisable information on outcomes for pregnant women and babies, although reported data favours the usage of Liposomal amphotericin B for the treatment of VL in pregnant women. Specific efforts should be made to enrol pregnant women in future clinical trials and a dedicated pregnancy registry should be constituted to prospectively collect data on the efficacy and safety of VL treatment during pregnancy.

Keywords VISCERAL LEISHMANIASIS, KALA-AZAR, SYSTEMATIC REVIEW, PREGNANCY

P2-019: PHARMACOKINETICS OF MILTEFOSINE AND PAROMOMYCIN IN A COMBINATION TO TREAT VISCERAL LEISHMANIASIS PATIENTS IN EASTERN AFRICA

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In Eastern Africa, effective, safe and affordable combination treatments for visceral leishmaniasis (VL) are still lacking. Geographical variability in paromomycin (PM) pharmacokinetics in Eastern Africa was previously observed, but drug exposure could not be related to treatment outcome. An allometric dosing regimen of oral miltefosine (MF) led to higher exposure than conventional dosing in paediatrics and more equivalent efficacy between children and adults. Recently, 14- & 28-day combination regimens of PM plus allometric MF were evaluated in Eastern Africa. In the current pharmacokinetic analysis, we aimed to characterize the pharmacokinetics of PM and MF in these combination regimens, to explore geographical pharmacokinetic differences, to evaluate adequacy of PM and MF exposure in children and adults, and to investigate exposure-response relationships for treatment outcome, toxicity and the development of PKDL. Pharmacokinetic data was collected in a multi-centre randomized controlled trial in VL patients from 6 sites in Kenya, Sudan, Ethiopia and Uganda. Patients received intramuscular PM (20 mg/kg for 14 days) plus oral miltefosine (MF) (allometric dose for 14 days (Arm 1) or 28 days (Arm 2). Sparse MF plasma samples were obtained in all patients in Arm 1 at D14

and in Arms 1 and 2 at D28 and D56. Intensive sampling was performed at D1 and D14 in a subset of patients from Kenya and Sudan. MF and PM concentrations were quantified using LC-MS/MS. Analysis was performed with a standard two-stage non-compartmental approach. PM exposure increased from D1 to D14, presumably due to decreased PM clearance over time. PM AUC_{0-24} appeared higher in adolescents/adults (>12 years) than in children (≤12 years), while C_{max} was similar. There was a trend of higher PM exposure in Sudan compared to Kenya, although this was not significant. PM exposure was higher compared to previous studies, suggesting adequate PM exposure. One patient had a 10-fold higher PM exposure on D14, associated with renal failure, which led to ototoxicity and permanent hearing loss. The allometric MF regimen led to equivalent MF exposures in children and adolescents/adults (e.g., 7% lower in children on D14 (arm 1)). No clear geographical differences in MF exposure were observed. The achieved median (RSD) Day 28 MF concentration in Arm 2 (25.9 µg/mL (26%)) and pharmacokinetic target achievement in children were even higher than previously reported for the allometric MF regimen in paediatric VL patients (21.0 µg/mL (16%)), confirming the suitability of the allometric MF regimen for children. No correlation could be identified between MF concentrations and development of relapse or PKDL indicating adequacy of the achieved drug exposure. Future studies will focus on a model-based pharmacokinetic analysis to further explore covariate analysis and exposure-response relationships.

Keywords VISCERAL LEISHMANIASIS; PHARMACOKINETICS; PAROMOMYCIN; MILTEFOSINE

P2-020: *Leishmania infantum* SELECTS CROSS-RESISTANCE TO ANTIMONY DERIVATES AFTER EXPOSURE TO THE IMMUNOSUPPRESSIVE AGENT METHOTREXATE

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Antimonials (Sb) are the traditional chemotherapeutic agents used to treat visceral leishmaniasis (VL). However, its effect is compromised by the rapid increase of resistance in *Leishmania* parasites. Nevertheless, VL treatment has less efficacy in immunosuppressed patients, among which are patients that receive immunosuppressant treatments, like methotrexate (MTX), to treat autoimmune disease. In order to analyse the growing problem these strains hinder in the VL treatment, this study aimed to determine the *in vitro* effect of Sb and MTX, as well as the interaction of both in different *Leishmania infantum* strains. Promastigotes of antimony and methotrexate resistant strains of *Leishmania infantum* were cultured for 72 hours with increasing concentrations of MTX or Sb^{III} to determine EC_{50} levels. As well, the EC_{50} of both drugs (MTX and Sb^{V}) in their amastigote form were determined after 5 days in infected Bone Marrow Derived Macrophages (BMDM). In addition, the Radical Oxygen Species (ROS) produced by promastigotes after 72h have been determined with the EC_{90} of both drugs. In the final step, co-cultures of promastigotes and EC_{90} of Sb^{III} or MTX for 5 days were made to determine cross-resistance profiles. Based on the reduction of viability of *L. infantum* promastigotes and amastigotes with increasing concentrations of the immunosuppressive agent, MTX had an antiparasitic effect. In addition, due to the significant increase in ROS

production, MTX had also an effect on the oxidative damage, compared to the control. However, the results suggested a cross-resistance effect between MTX and Sb based on the statistically significant increase in the EC_{50} and the significant decrease in ROS produced, after contact with Sb^{III} and MTX, respectively. In conclusion, methotrexate induces *in vitro* cross-resistance of *L. infantum* against antimony that could pose an added difficulty in the treatment of leishmaniasis in patients with autoimmune diseases treated with methotrexate.

Keywords ANTIMONIALS; METHOTREXATE; DRUG RESISTANCE

P2-022: TREATMENT OF CUTANEOUS LEISHMANIASIS IN THE ELDERLY WITH LIPOSOMAL AMPHOTERICIN B: A RANDOMIZED CLINICAL TRIAL

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Cutaneous leishmaniasis (CL) is an important public health problem in Brazil, caused mainly by *Leishmania (Viannia) braziliensis*, representing more than 90% of the total cases. CL is predominantly found in adult males exposed to forest regions. In recent years the epidemiology of CL has changed, affecting also women, children, and the elderly. CL in the elderly results in a therapeutic challenge, since pentavalent antimony (Sb^v) is not recommended in this age group where heart, liver or kidney disease are commonly found. In this context, liposomal amphotericin B may become an attractive systemic therapy in this group, due to potential less toxicity and better efficacy. However, there is little experience and a lack of data in the literature regarding its use in CL and the better low dosage associated with few toxicity and good efficacy. The present study was a randomized controlled and double blinded trial aimed to identify the dose of liposomal amphotericin B associated with the highest cure rate in the elderly. Thirty-two patients from the endemic area of Corte de Pedra, Bahia, Brazil aged 60 years or older of both genders, with localized and ulcerated CL were included. Diagnosis was confirmed upon a positive PCR for *L. braziliensis* in tissue obtained from ulcers. The groups were treated with liposomal amphotericin B (AmBisome®) with three different total doses after randomization: Group 1 (G1) received a total dose of 12 mg/kg (10

patients). Group 2 (G2): 18 mg/kg (10 patients). Group 3 (G3): 24 mg/kg (12 patients). The drug was used twice a week in an outpatient hospital care. Clinical and laboratory assessments were performed before the start of therapy (D0), and at D15, D30, D60, D120 and D180. The average ages of groups G1, G2 and G3 were: 68.5; 72.5 and 67.4 respectively. The number of lesions ranged from 1 to 3 (median 1.5) with no differences between the groups. The lowest healing time (days) was 57.0 (G3) compared to 92.5 (G1) and 67.5 (G2). Failure rates in groups G1, G2 and G3 were 15%, 37.5% and 0% respectively. Regarding side effects, there were no differences between the groups; mild and transient raised levels of creatinine and/or BUN were documented in less than 30% of subjects. Only one patient (G2) interrupted therapy due to anaphylaxis that was controlled. CL in the elderly represents a therapeutic challenge in *L. braziliensis* endemic regions due to its aggressivity, contra indication of Sb^v, and potential toxicity for deoxycholate amphotericin B. When systemic therapy is indicated and miltefosine is not available or fails, liposomal amphotericin may be a good option. There is a lack of trials with this drug in cutaneous and mucosal leishmaniasis, mainly in the elderly. Our trial contributes to indicate a safe and effective total dosage in CL treatment in this age group. The total dosage of 24 mg/kg has a high rate of cure and is safe for the treatment of CL in the elderly.

Keywords CUTANEOUS LEISHMANIASIS; LIPOSOMAL AMPHOTERICIN B; ELDERLY; *L. braziliensis*

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P2-023: ESTIMATING THE PROPORTION OF RELAPSE FOLLOWING TREATMENT OF VISCERAL LEISHMANIASIS (VL): META-ANALYSIS USING IDDO LIVING SYSTEMATIC REVIEW

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Following treatment of Visceral Leishmaniasis (VL), patients are followed-up for at least 6-months to capture potential relapse. Relapses after 6-months have been reported in observational cohorts leading to suggestion that a longer follow-up may be warranted. A meta-analysis was carried out to quantify the proportion of relapses at 6-months and 12-months of follow-up using the Infectious Diseases Data Observatory (IDDO) systematic review database of VL clinical studies. Studies indexed in the IDDO VL living systematic review of clinical studies were screened (1983-2021; 182 studies). Only studies with a minimum follow-up of 6-months that clearly reported relapse (or absence) were included; studies in VL-HIV coinfection were excluded. A meta-analysis of single proportion was carried out to obtain a pooled estimate of relapse; sub-group analyses were carried by drug regimen, total mg/kg of Liposomal Amphotericin B (L-AmB), follow-up duration, and geographical region. Heterogeneity was quantified using I^2 statistics. Estimates from random effects meta-analysis were reported together with (95% confidence interval, CI). A total of 120 studies (27,902 patients) were included in the meta-analysis; 95 (79%) were from the Indian Sub-continent (ISC), 12 (10%) from East Africa (EA), 4 (3%) from the Mediterranean region, 4 (3%) from Latin America, 3 (3%) from central Asia, and 2 (2%) were multi-regional. Overall, 25,911 patients were initially cured and 2,149 of them relapsed. In the studies from the Indian

subcontinent (ISC), the estimate of relapse at 6-months was 3.9% [95% CI: 2.8%-5.4%; I^2= 88%] and when stratified by drug regimen the estimates were: 8.6% [95% CI:4.1%-17.2%] for Pentavalent Antimony (PA), 3.4% [95% CI: 2.4%-4.9%] for single dose L-AmB, 1.2% [95% CI: 0.3%-4.6%] for L-AmB in a combination regimen, and 0.7% [95% CI:0.2%-2.6%] for miltefosine and paromomycin. In the ISC, the estimate of relapse following single dose L-AmB stratified by mg/kg dosage were: 6.7% [95% CI: 1.4%-18.2%] among those receiving 3-5 mg/kg, 3.5% [95% CI: 2.3%-5.2%] for 5-10 mg/kg and 2.0% [95% CI: 0.6%-5.9%] for 10-15 mg/kg (*P*=0.083). In East Africa, the overall estimate of relapse was 9.3% [95% CI:5.7%-14.8%; I^2= 71%] and the estimates varied by drug regimen: 6.9% [95% CI: 1.7%-24.3%] for single dose L-AmB in a combination regimen, 9.0% [95% CI:3.0%-23.9%] for PA, 6.3% [95% CI: 0.9%-33.5%] for Paromomycin, 10.7% [95% CI:3.7%-28.0% for miltefosine, and 12.9% [95% CI: 4.4%-32.9%] for PA and Paromomycin regimen. From 19 studies that reported relapses at 6- and 12-months, the proportion of relapse was 0.5% [95% CI: 0.1%-2.3%] at 6-months and 1.5% [95% CI: 0.7-3.8] at 12-months, with a pooled absolute underestimation of relapse by 1.0% [95% CI: 0.6%-1.0%] at 6 months.This review estimated that approximately 5-10% of patients relapsed following treatment of VL with large heterogeneity in the estimates. Combination regimens had lower relapse rates over monotherapies. Limited data from studies with longer follow-up suggested conventionally adopted 6-months period leads to an underestimation of relapse. A closer examination using individual patient data hosted at IDDO platform is warranted to gauge the optimal follow-up duration..

Keywords VISCERAL LEISHMANIASIS; KALA-AZAR; SYSTEMATIC REVIEW; RELAPSE; EFFICACY; META-ANALYSIS

P3-011: DYNAMICS OF THE CLINICAL AND IMMUNOLOGICAL FEATURES ON HUMAN *Leishmania (L.) infantum chagasi* INFECTION IN SOUTH HONDURAS

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In Honduras, Central America, *Leishmania* (*L.*) *infantum chagasi*-infection leads to atypical clinical manifestations in humans, causing non-ulcerated cutaneous leishmaniasis (NUCL) in adolescents and young adults, and visceral leishmaniasis (VL) in children under five years old. Recently, we described a high prevalence (73.6%) of human infection in Isla del Tigre, Amapala municipality, southwest of Honduras; and based on the clinical, parasitological and immunological diagnosis we characterized seven profiles of infection, three asymptomatic [Initial Asymptomatic Infection - IAI (ELISA+/DTH-), Resistant Asymptomatic Infection - RAI (ELISA+/DTH+), Final Asymptomatic Infection – FAI (ELISA-/ DTH+)] and four symptomatic [Early Symptomatic Infection - ESI (ELESA-/DTH-), Initial Symptomatic Infection - ISI (ELISA+/DTH-), Resistant Symptomatic Infection - RSI (ELISA+/DTH+), Final Symptomatic Infection - FSI (ELISA -/DTH+)]. However, the lack of reports on the evolution of NUCL and the increase number of cases, lead us to study the dynamics of the clinical-immunological evolution of infection in that region. We followed up a cohort of 576 individuals for a two years period through clinical, parasitological

and immunological evaluations. After clinical examination, the individuals were submitted to Montenegro skin test (DTH) with homologous antigen and total blood was collected for IgG and IgM detection in sera by ELISA. NUCL cases were confirmed by skin parasite search and PCR-RFLP characterization of the parasite. In the prevalence, we observed 82% asymptomatic and 18% symptomatic infection only by NUCL. All NUCL cases showed a positive parasitological diagnosis and *L.* (*L.*) *infantum chagasi* was confirmed. During the incidences, 26.4% of asymptomatic individuals (ELISA-/DTH-) converted to IAI (ELISA+), 3.7% to RAI (ELISA+/DTH+) and 24% to FAI (DTH+) profiles. Individuals belong to IAI (ELISA+) profile in the prevalence, 26.2% converted to RAI (ELISA+/DTH+) and 17.1% to FAI (DTH+) profiles; and the most of RAI move towards the FAI profile. It is important to note that no case of FAI developed skin lesions suggestive of NUCL. Concerning to symptomatic infection (NUCL), FSI (DTH+) was the most frequent profile (30.8%) observed in the prevalence. From ESI profile, characterized by skin lesion with positive parasitological diagnosis but with negative immunological tests, 59% of them converted to FSI (DTH+), 18% to RSI (ELISA+/DTH+) and 18% to ISI (ELISA+) profile. Regarding the ISI (ELISA+), 10% converted to RSI (ELISA+/DTH+) and 25% to FSI (DTH+) profiles. Finally, the most of RSI converted to FSI profile. It is important to note that during all study period, none of LCNU cases developed visceral disease. The evolution of asymptomatic and symptomatic infection showed the predominance of FAI and FSI profiles, pointing to a profile of resistance of human infection in the inhabitants of the Pacific region of Honduras.

Keywords NON-ULCERATED CUTANEOUS LEISHMANIASIS; *Leishmania* (*Leishmania*) *infantum chagasi*; HUMORAL IMMUNE RESPONSE; CELLULAR IMMUNE RESPONSE; HONDURAS

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P3-012: VARIATIONS IN THE CLINICAL PRESENTATION OF LEISHMANIASIS WITH MUCOSAL INVOLVEMENT

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Although it is recognized as one of the most disfiguring forms of leishmaniasis and a late complication, especially relevant in Americas, leishmaniasis with mucosal involvement (LMI) is still surrounded by scarce scientific evidence and lacks a comprehensive operational definition. The lack of a standardized and widely accepted classification prevents the comparison of studies, harming the construction of the evidence needed to guide the disease approach and prognostic assessment. This study aims to identify demographic, clinical, laboratory and prognostic aspects capable of distinguishing the different clinical presentations for LMI. Between April 2017 and September 2020, 124 patients with mucosal manifestation and leishmaniasis suspicion were recruited at a referral centre in Brazil. Patients with active mucosal lesions associated with *Leishmania* infection confirmed by direct or histological examination, culture, or polymerase chain reaction or, alternatively, by therapeutic response, ruled out other diagnoses, were considered true ML cases. Out 102 patients with confirmed ML, 50 (49%) had exclusive mucosal involvement while other 52 (51%) had lesions in the mucosa and skin concomitantly. Despite most patients had cutaneous and mucosal involvement simultaneously, in 78.8% of cases the affected skin was contiguous to mucosa. The predilection of leishmaniasis by the nasal mucosa was confirmed, present in 89.2% of cases. The number of skin lesions ranged from 1 to 11 (excluding three cases with countless lesions); 28 (53.8%) of the 52 patients had a single skin lesion and eight (15.4%) patients had more than six lesions. Patients presenting exclusive mucosal lesions exhibited longer disease duration, higher proportion of cases with involvement restricted to the nose and more severe forms. On the other hand, cases with both cutaneous and mucosal involvement had a higher rate of oral lesions and positivity in the immunohistochemical study. Patients

with mucosal involvement restricted to the upper aerodigestive tracts (with or without contiguous skin lesions) had a higher rate of therapy failure at 6-month follow-up, compared to patients with other clinical presentations (16.7% vs 3.5%). The main observation of this study was the confirmation of different LMI presentations concerning to demographics, clinical and prognostic aspects: one form restricted to the nose/respiratory system above the glottis/throat, with or without involvement of the adjacent skin, characteristic of older patients, with longer disease evolution and worse prognosis. Patients with mucosal involvement in the context of multiple skin lesions, were younger than patients with lesions restricted to the nose. Patients with skin lesions distant from the mucosal involvement site exhibited a higher proportion of ulcerated-type skin lesions and a higher probability of a positive immunohistochemical result, with a shorter time of disease evolution compared to patients with disease restricted to aerodigestive mucosa. If confirmed by other studies and in larger series, these findings can in future compose algorithms for diagnostic and prognostic evaluation for LMI.

Keywords MUCOSAL LEISHMANIASIS; CLINICAL CLASSIFICATION; DIAGNOSIS

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P3-013: LATE MUCOSAL COMPLICATION IN PATIENTS TREATED FOR CUTANEOUS LEISHMANIASIS: A RETROSPECTIVE COHORT STUDY

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Although it can also occur concomitantly to the skin lesions, mucosal involvement typically occurs years after cutaneous leishmaniasis (CL). Representing about 3% to 6% of all CL cases annually registered in Brazil, mucosal leishmaniasis (ML) has a high potential to cause functional abnormalities, deformities, and permanent sequelae. Several risk factors have been suggested as related to the occurrence of ML, such as the absence, late or inadequate treatment for CL. The influence of other risk factors, such as the extension and location of the primary cutaneous lesions (upper body and face) and patients' clinical characteristics (age, comorbidities, immunosuppression) have also been implicated. In this study, the rate of mucosal involvement after different treatment regimens for CL was assessed. A retrospective cohort study involving 226 new CL cases, reported between 2015 and 2018, was carried out at a referral centre for CL in Brazil (Fiocruz Minas). All medical records were analyzed, and an active search of all cases was performed through telephone contact looking for signs and symptoms compatible with ML. Patients who reported complaints compatible with mucosal lesions according to a structured and comprehensive questionnaire were then submitted to a clinical and to an otorhinolaryngological exam aiming at confirming the presence of mucosal involvement. Among the 176 CL cases eligible for this cohort, 174 (98.8%) had specific treatment for CL, the other two had skin lesion spontaneous healing. Treatment for CL was defined according to the Brazilian guidelines, based on the patients' clinical characteristics, at medical discretion. The therapeutic regimen for CL was meglumine antimoniate intravenously in 72

cases (41.4%), intralesional infiltration of meglumine antimoniate in 97 cases (55.7%) and liposomal amphotericin B in five patients (2.9%). The intralesional infiltration of meglumine antimoniate regimen used was based on a median of five infiltrations per patient (IQ25-75% 3-6) and median volume total drug infiltrated along the treatment of 25.5 mL (IQ25-75% 14.3-41.0) and a median duration of 43 days (IQ25-75% 30-62.5 days). The median follow-up time was 38.5 months (IQ25-75%:35-46), ranging from 3 to the maximum 5.5 years after the treatment for CL. Four patients evolved with mucosal complication, a rate of 2.3% (95%CI: 0.90-5.73). The median time to confirmation of the mucosal complication was 13.5 (IQ25-75%: 7.3-22.8) months after CL treatment. In all four cases, the mucosal involvement reached the nasal mucosa, all patients had been treated with meglumine antimoniate intralesional infiltration. The primary CL manifestation was a single, ulcerated lesion in the upper body in all four patients. This is a small cohort study without statistical power to identify prognostic markers to late complications. However, these observations alert to the occurrence of late mucosal involvement among patients undergoing local treatment with of meglumine antimoniate intralesional infiltration, which requires further long-term studies.

Keywords CUTANEOUS LEISHMANIASIS; RISK FACTORS; INTRALESIONAL THERAPY; FOLLOW-UP; MUCOSAL LEISHMANIASIS

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P3-014: ATYPICAL AND RELAPSING CUTANEOUS LEISHMANIASIS AFTER COVID-19 IN A PATIENT WITH TREATED MYCOSIS FUNGOID

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Atypical manifestations of cutaneous leishmaniasis (CL) may be triggered by alterations in the host immune response. We report an atypical and relapsing case of CL after a severe SARS-CoV-2 pneumonia in a patient with previously treaded mycosis fungoid (MF). A 78-year-old male resident of a leishmaniasis endemic region in Brazil had 8-year prior MF, biopsy confirmed, with exclusive manifestation in back hands. He was successfully treated only with topical betamethasone BID and kept using it several times along the years. Seven years later, patient presented an ulcerated lesion on the right-hand's dorsum, biopsy diagnosed as CL and treated with 2g lipossomal amphotericin B healing completely. Two months later, he had SARS-CoV-2 severe pneumonia and received 20mg dexamethasone intravenous for 5 days followed by 10mg 5 days among other support drugs in ICU. After 27 days, he was discharged with complete resolution of the pulmonary condition. One month after hospital discharge, patient presented an infiltrated erythematous plaque on previous LC lesion site, that evolved to an ulcer and multiple erosions. He received 4g of lipossomal amphotericin B, but the ulcer didn't heal, and a new biopsy was made. It showed intense lymphomononuclear infiltrate with polymorphonuclear cells and mastocytes in the dermis and small granulomas permeated by multinucleated giant cells. The parasite survey showed small globoid shapes, containing kinetoplasts. The immunohistochemical assay was

positive for *Leishmania spp*, T-lymphocyte CD3+, cytokeratins and rare for B-lymphocyte CD20+. The PCR test was also positive for *Leishmania spp*. Thus, CL was confirmed, and MF recurrence excluded. The patient was treated with 8 weeks oral combined therapy with miltefosine 50mg BID and pentoxifylline 400mg TID and pentoxifylline alone for other 8 weeks. After 16 weeks of treatment all lesions completely healed. The COVID-19 pandemic brought the discussion of host immune response and infection mechanism to the spotlight. In this context, co-infections and immunosuppression are particularly intriguing. Our patient had MF treated with topical corticosteroid which he used irregularly several times. It is known that topical corticosteroids chronical use can cause local immunosuppression, facilitate parasitological infections and it can favor atypical and disseminated manifestations. This patient also used systemic corticoid to manage COVID-19 pneumonia that could facilitate CL recurrence. Also, CL and MF can mimic each other, but immunohistochemical assay and parasite demonstration may distinguished them. SARS-CoV-2 infection has a wide range of clinical manifestations and severity. The cytokine storm, responsible for the disease severity and mortality, is associated with the immune activation Th1 pathway. Typically, localized CL is also associated to Th1 pathway increase, while diffuse CL is linked to Th2 pathway. Additionally, some lateral-flow immunoassays and enzyme-linked immunosorbent assays for detecting anti-SARS-CoV-2 antibodies had significant false-positive results in leishmaniasis patients. This indicates that antibodies may have similarities and the immune memory may influence disease manifestations. Corticosteroid's immunosuppression can influence LC recurrence and clinical forms and there are many unanswered questions about the immunological mechanisms of both SARS-CoV-2 infection and CL, but some common pathways may be involved and interfere in clinical manifestations and response to treatments.

Keywords ATYPICAL CUTANEOUS LEISHMANIASIS; COVID-19; CORTICORSTEROID; MILTEFOSINE; MYCOSIS FUNGOID

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P3-015: ASYMPTOMATIC *Leishmania* INFECTION IN HIV-POSITIVE OUTPATIENTS ON ANTIRETROVIRAL THERAPY IN PERNAMBUCO, BRAZIL

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Visceral leishmaniasis (VL) in HIV-positive individuals is a global health problem. HIV-*Leishmania* coinfection worsens prognosis and mortality risk, and HIV-*Leishmania* coinfected individuals are more susceptible to VL relapses. In addition, asymptomatic *Leishmania* infected individuals, despite the typically low parasite load, might contribute to maintaining the transmission cycle of *Leishmania* parasites in endemic regions during episodes of increased parasite load and disease relapse. Therefore, early initiation of antiretroviral therapy can protect against *Leishmania* infection in individuals living in VL-endemic areas, and regular use of antiretrovirals might prevent VL relapses in these individuals. We conducted a cross-sectional study in Petrolina, Brazil, an VL-endemic area, to estimate the prevalence of asymptomatic *Leishmania* cases among HIV-positive outpatients. We invited any HIV-positive patients, aged ≥ 18-years-old, under antiretroviral therapy, and who were asymptomatic for VL. Patients were tested for *Leishmania* with enzyme-linked immunosorbent assays (ELISA)-rK39, immunochromatographic test (ICT)-rK39, direct

agglutination test (DAT), latex agglutination test (KAtex), and conventional polymerase chain reaction (PCR). HIV-*Leishmania* coinfection was diagnosed when at least one VL test was positive. A total of 483 patients were included. The sample was predominantly composed of single, < 48-years-old, black/*pardo*, heterosexual males, with fewer than 8 years of schooling. The prevalence of asymptomatic HIV-*Leishmania* coinfection was 9.11% (44/483). HIV mono-infected and HIV-*Leishmania* coinfected groups differed statistically significantly in terms of race (p = 0.045), marital status (p = 0.030), and HIV viral load (p = 0.046). Black/*pardo* patients, married patients, and those with an HIV viral load up to 100,000 copies/ml presented higher odds for HIV-*Leishmania* coinfection. A considerable number of asymptomatic *Leishmania* cases were observed among HIV-positive individuals in a VL-endemic area. Given the potential impact on transmission and health costs, as well as the impact on these coinfected individuals, studies of asymptomatic *Leishmania* carriers can be useful for guiding public health policies in VL-endemic areas aiming to control and eliminate the disease.

Keywords VISCERAL LEISHMANIASIS; HIV; COINFECTION; ASYMPTOMATIC

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P3-016: USING 3-DIMENSIONAL SCANNING TO OBJECTIFY CHANGES OF CUTANEOUS LEISHMANISIS LESIONS

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Clinical studies to evaluate which cutaneous leishmaniasis (CL) treatment option is best require a good clinical reference standard to assess treatment response objectively. Although it is recommended that outcomes are assessed based on clinical assessment of re-epithelization and flattening, assessment of cure for CL lesions is often variable across clinicians and difficult to standardize. A first report showed that volume and size of papules of post-kala azar lesions can be assessed using a 3D-scanner. Similarly, 3D-scanners may decrease inter-observer variability for outcome assessment in CL. As a first step, we carried out a small pilot project to see whether 3D-scanning of CL lesions is feasible, and whether changes in color, texture, and geometry can be captured over time. Patients who were clinically or parasitologically diagnosed as having CL were recruited at Boru Meda Hospital, Ethiopia in February and May 2021. They were scanned with the Artec Eva 3D scanner at recruitment (BL) and at follow-up-up (3-7 days after recruitment (W1), and after 3 months for 3 patients (M3)). Healthy controls, recruited amongst hospital staff and patient family members, were also scanned, with 7 days between scans. Intra-patient scan differences were visualized by heatmaps that represented Euclidean distance, mean curvature dissimilarity and ΔE* color distance. Repeat scans were available for 18 patients, of which 15 had W1 scans at least 7 days apart, and 3 had M3 scans at least 3 months apart. For every patient, the region of interest (ROI) was manually selected. Most patients had lesions on the nose, cheek, or lips. For healthy volunteers, 13 W1 scans were made. In 67% of the cases,

a decrease of curvature in the ROI was noticeable after one week. Most of these patients had nodular lesions and swelling. In two patients with M3 scans (nodular lesions), this difference became more prominent. For all patients, the mean difference between baseline (BL) and W1 was 7%; the difference between BL and M3 was 13% for the three patients. In 67% of all cases, a decrease of color difference in the ROI was noticeable after one week; more pronounced differences were detected in patients with crusted and plaque-like lesions. Overall, the difference in color between scans at two time-points was more subtle, as the mean difference between BL and W1 was only 4%. Lesion differences on lips, around the nostrils and eyes were difficult to detect, as even in healthy volunteers, differences over time were detected due to facial expression. 3D-scanning for CL can be useful for objectively comparing lesion progression over time using two timestamps. Analysis of the scans showed that one week seems too short to detect differences, although visualizing differences at month 3 seems more promising. For nodular lesions, curvature and distance look most useful to objectify lesion flattening, but for crusted and plaque-like lesions, color changes seem more suitable parameters to objectify lesion progression. Noise, e.g. due to facial expression, can be reduced by defining the affected region and type of lesion beforehand and by comparing only the ROIs.

Keywords OUTCOME ASSESSMENT; 3D SCANNING; 3D MODELS; MEDICAL IMAGING

P3-018: ECG CHANGES IN VISCERAL LEISHMANIASIS PATIENTS IN ETHIOPIA: PREVALENCE AND RISK FACTORS

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Visceral leishmaniasis (VL) is fatal if left untreated. Pentavalent antimonials are still commonly used as VL treatment and are part of the first line treatment in Ethiopia. In many resource-constrained settings, these drugs are often given without baseline and follow up ECG monitoring. However, antimonials have been reported to display cardiotoxic effects, including QTc prolongation. To what extent VL in itself can lead to ECG abnormalities is less well documented. We conducted a prospective study to determine the prevalence of ECG changes at baseline (after VL diagnosis, before treatment) and at the end of treatment. Additionally, we defined which patients are most likely to display ECG changes and hence would benefit from ECG monitoring. All non-pregnant VL patients treated with any kind of VL regimen between November 2019 and November 2020 at the Leishmaniasis Research and Treatment Center in Gondar, Ethiopia were included. Sociodemographic, clinical, laboratory and ECG data were captured at baseline and at end of treatment. ECGs, done at baseline, end of treatment and as needed in between, were reviewed by cardiologists. Risk factors for ECG changes were determined using logistic regression. At the time of submission of the abstract, 144 VL patients had been included. Of these, 146 were male; the median age was 23 years (IQR 20-27). Three were known with chronic cardiac disease and six had HIV coinfection. The median body mass index was 16.6 Kg/m^2. The median baseline hemoglobin level was 8.5 g/dL. 74 (51%) were treated with antimonials/paromomycin combination therapy, 44 (30%) with miltefosine/paromomycine combination, 10 (7%) with antimonial monotherapy, 11 (8%) with AmBisome monotherapy, and

5 (3%) with AmBisome/miltefosine combination therapy. At baseline, ECG abnormalities were present in 46 (32%) of the 144 VL cases. T-wave changes (n=19; 13%) were the most common abnormality, followed by QTc prolongation (n=12; 8%), intraventricular conduction abnormalities (n=9; 6%), first-degree AV-block (n=9; 6%), PR interval prolongation (n=9, 6%) and ST-T changes (n=2; 2%). At the end of treatment, ECG abnormalities were present in 20 (15%) amongst the 130 patients with ECG available at the end of treatment. The frequency of all baseline ECG abnormalities was reduced, with T wave changes (n=15; 11%), QTc prolongation (n=8; 5%) and intraventricular conduction abnormalities (n=5; 4%) remaining the most common. For ECG abnormalities at baseline, anemia, jaundice and a low body mass index were identified as independent risk factors. ECG abnormalities at the end of treatment were most commonly seen in those with anemia but were not more frequent for those receiving antimonial treatment. Risk factors for QTc prolongation (QTc > 450msec) included anemia, but no increased risk was seen for those treated with antimonials. ECG changes were frequent at the start of treatment but were clearly less common at the end of treatment. Use of antimonials was not associated with an increased prevalence of ECG abnormalities. The frequency of QTc prolongation was also decreased after treatment, and not associated with the use of antimonials. While our preliminary data are reassuring, a formal re-assessment of this remains to be done after full enrolment in the study.

Keywords VISCERAL LEISHMANIASIS; ECG; PENTAVALENT ANTIMONIALS; CARDIOTOXICITY

P3-019: DISSEMINATED CUTANEOUS LEISHMANIASIS BY *LEISHMANIA PANAMENSIS*: A CASE SERIES

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American Cutaneous Leishmaniasis (ACL) is a parasitic disease caused by several species of the genus *Leishmania*. One of its clinical forms is Disseminated Leishmaniasis (DL), a severe and emerging presentation which is characterized by the presence of multiple papular acne-like lesions affecting different segments of the body. The number of lesions can reach several hundred beginning with one or more lesions with the classic characteristics of granulomatous ulcers with raised borders, and mucosal involvement described in half of the cases. DL development is poorly understood and is related to a complex network involving environmental, host immune response, and parasite factors. *Leishmania* is an obligate intracellular parasite, mainly of macrophages which after cutaneous inoculation by sandflies (*Lutzomya*), phagocytose the flagellated form (promastigote) that differentiates into the amastigote form. Local production of TNF-α and IL-12 drives the immune response to the secretion of Th1 cytokines such as IFN-γ and others, which activate the macrophage to kill *Leishmania*, however, a decreased in peripheral Th1 response in DL patients has been described, allowing for the spread of the parasite and its clinical presentation. Here, we present two male patients, a 44-year-old farmer from Cerro Azul, Panama City and a 29-year-old police officer working in Cocoli, Panama Canal Zone, who presented on physical

examination with 63 and 52 polymorphic lesions respectively, distributed in the facial area, nasal mucosa, trunk, and extremities. Scraping samples for smear, PCR (Polymerase Chain Reaction) and culture were taken, Montenegro test is also applied to them, which are reported as positive, after which the diagnosis of DL with nasal mucosa involvement. The parasite was characterized as *Leishmania (Viannia) panamensis* by the Hsp70-RFLP methodology using the enzymes HaeIII and BccI. Biopsy samples were taken to carry out immunohistochemical and histopathological analyses, observing the presence of amastigotes in moderate quantity and the presence of histopathological alterations in the epidermis and dermis. The description of these cases allowed us the opportunity to perform flow cytometric and immunohistochemical tests, a cytometric analysis comparing 3 healthy donors vs *Leishmania* infected patients show an increase in the monocyte (CD14) cell subset, meanwhile a decrease in mature B cells (CD19), as per cytotoxicity activity, CD8 T cells were compared with different stimulation conditions (unstimulated, IL-18/IL-12, PMA/ionomycin) to evaluate cell response, showing an increase of TNFa/IFNgamma production upon different stimulation compared to controls; thanks to which a description of the host's immune response to the parasite can be provided, before receiving treatment, thus contributing to a better understanding of this rare presentation of Cutaneous Leishmaniasis.

Keywords DISSEMINATED LEISHMANIASIS; *Leishmania panamensis*; PANAMA; CUTANEOUS; IMMUNE RESPONSE

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P3-021: DIFFUSE LEISHMANIASIS CAUSED BY *Leishmania (Leishmania) amazonensis* IN PERU. A CASE REPORT

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Leishmaniasis is a neglected tropical disease with broad cutaneous clinical forms, being most frequently the localized cutaneous. Conversely, a clinical form low reported is diffuse cutaneous, characterized by present papules, nodules, infiltrated plaques that could to affect broad areas of body. This variation occurs due to interaction of multiples factors, mainly the Leishmania species involving and the immunity status of patients. This report, we described a case of an older adult that develop Diffuse Cutaneous Leishmaniasis in a long time. Patient of 61 years old, masculine, from locality of San Francisco, province of La Mar, department of Ayacucho, Peru. Who refers that in January 2008, began disease with appearance of papular lesions in right arm, left thigh and face, which increased in size over time, spreading throughout the body. The patient goes to the Ayacucho Hospital where the disease was confirmed by microscopy, in addition presented positive result in the IFA. The patient was administered a cycle of sodium stibogluconate, at the end of treatment he did not attend the control and the follow-up was lost. In September 2013, the patient returned to the Hospital where was newly administered sodium stibogluconate was for 30 days, obtaining a low response to treatment. Five months later, the patient was hospitalized in the same nosocomium, for the administration of the second-line treatment amphotericin B deoxycholate, without improvement, so he is referred to the city of Lima, to Cayetano Heredia National Hospital for better clinical management, where amphotericin B deoxycholate was administered; however, the patient abandoned treatment. In March 2016, the patient was admitted to the Hipólito Unanue National Hospital, finding

nodular lesions with a diameter of approximately 25 to 30 mm each nodule in approximately 85% of the body, involving the entire face, ears, trunk, upper and lower limbs. In addition, some lesions at the level of the upper limbs presented purulent discharges for this he's referring a mild pain. The National Leishmaniasis Reference Laboratory of the INS-Perú confirmed by microscopy and vitro Culture, likewise, the presence of circulating anti-Leishmania IgG antibodies at titer of 1/1280 was detected by means of the IFA and a negative response to the Montenegro skin test. By sequencing the cytochrome B gene, *Leishmania (Leishmania) amazonensis* was identified. In addition, ELISA test for HIV and HTLV was performed with negative results. The patient was administered intravenously Amphotericin B deoxycholate (0.8 mg/Kg/day) for 30 days; when observing the clinical improvement of the skin lesions and presenting episodes of hypokalaemia forced to interruption the treatment, on several occasions. He also developed nosocomial pneumonia; a situation that worsened his clinical condition and he died. This case illustrates the aggressiveness of diffuse cutaneous leishmaniasis caused by *Leishmania amazonensis*. We believe it is necessary to determine the prevalence and magnitude of this clinical form in Peru. This clinical form in most cases has a low response to first and second line treatment, being urgent to evaluate effective alternative therapies for the treatment and control of this severe form of the disease.

Keyword DIFFUSE CUTANEOUS LEISHMANIASIS; AMPHOTERICIN B; *Leishmania amazonensis;* PERU

P3-023: CUTANEOUS LEISHMANIASIS PRESENTING SYSTEMIC SYMPTOMS: A CASE REPORT

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American Cutaneous Leishmaniasis (ACL) is a disease transmitted to humans by the bite of phlebotomine sandflies, caused by flagellate protozoa of the genus *Leishmania*. In Brazil, seven species of *Leishmania* responsible for ACL in humans have been identified. Cutaneous leishmaniasis is the most common form of leishmaniasis and is characterized by ulcerated skin lesion without systemic symptoms. Realized parasitological diagnosis with findings of the etiological agent microscopically, culture and the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) exam for *Leishmania* in cutaneo lesion suggestive of leishmaniasis at infectious disease referral service in state of Mato Grosso. A 73-year-old patient sought the service of the Universitary Hospital Júlio Muller in September 2020, complaining of afternoon fever (38.5°) for about 1 month, associated with indisposition, weight loss (3 kg in a month), hoarseness and crusted lesion on the left eyebrow with subcutaneous infiltration, with progressive increase in diameter, and the presence of a palpable lymph node in the preauricular and ipsilateral frontotemporal regions. The patiente had serologies for sexually transmitted infections and negative rheumatologic markers. The culture and the direct search for *Leishmania* were positive in the lesion biopsy, confirming the diagnosis of American Tegumentary Leishmaniasis. In addition to the PCR-RFLP exam confirmed the *braziliensis* specie. Treatment was prescribed with Liposomal Amphotericin, 50 vials, accumulated dose of 31.25mg/kg/day for 12 days with indication because the age over 60 years. After the end of the treatment, there was maintenance of the clinical picture with worsening of the lesions and the emergence of a new typical lesion on the knee. In which it was indicated a new cycle of treatment with pentamidine 300 mg, 5 doses, with clinical improvement and

partial healing of lesions. After 5 months, there was a reappearance of phlogistic signs in the lesion and a return of fever. Then, 3 more doses of Pentamidine were prescribed, resulting in an improvement in the clinical picture and total healing of the lesions. The scientific importance of this case report it's due to American Cutaneous Leishmaniasis with localized ulcer and exhibited systemic symptoms in an immunocompetent patient. Moreover, require complementation of the therapeutic owing to recurrence of the lesions after the end of the initial pre proposal with Liposomal Amphotericin B.

Keywords *Leishmania*; PENTAMIDINE; AMPHOTERICIN

P3-024: MUCOCUTANEOUS LEISHMANIASIS IN PARAGUAY: FACTORS CONTRIBUTING TO THE BURDEN OF THE DISEASE HAMPERING ADEQUATE DIAGNOSIS

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Worldwide, leishmaniasis is among the ten most neglected tropical diseases, with the mucosal form (ML) being an endemic problem in rural populations in Paraguay. Diagnosis is complex, based on combining costly laboratory methods that are performed only in large cities and that include lesion biopsies and molecular techniques. In our country, there are few specialists trained in recognizing cases and collecting samples. The objective of this work was to determine the factors that limit and/or hinder the achievement of the diagnosis of ML by direct methods in Paraguay. And how it affects the burden for leishmaniasis cases. Descriptive observational study based in two pilot studies: a questionnaire in 20 patients (2017) concerning their perception of service received in public institutions during the diagnosis, and another carried out in 17 suspected cases of ML in the Department of Tropical Medicine of the IICS, in the period 2018/19, who were referred from the University Hospital. It was noted in both surveys that availability of appropriate diagnostic methods, especially regarding ML, relies on circumstantial factors, such as bureaucracy, political situation, financial restraints at the Ministry of Health (MH). Access to direct diagnostic methods for ML to a very limited fraction of the population is a pathetic sign of the inequity of opportunities in health for the population. It is also serious that the diagnostic guidelines are not permanent at the MH, so they become confusing for health technicians. The weak scheme of the MH regarding

leishmaniases in Paraguay is critical, if considered the large investment made by public funds on the disease. Regarding ML cases themselves, the burden is huge, but their condition should necessarily be improving soon. Otherwise it would become a crisis of human rights.

Keywords LEISHMANIASIS; MUCOCUTANEOUS; BURDEN; SURVEY; DIAGNOSIS

P3-025: FIRST REPORT OF *Leishmania infantum* IN MILK IN A NATURALLY INFECTED BITCH FROM BRAZIL.

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Visceral Leishmaniasis is a cosmopolitan disease, caused by *Leishmania infantum* affecting humans and several animal species. Among animals, dogs are the main known reservoir and play an important role in transmission cycle through infected sandflies, particularly *Lutzomyia longipalpis* in Brazil. Dogs, besides reservoirs, are also susceptible to infection and often get sick and even die. The amastigote forms spread through in the body and was already described in the bone marrow, blood, spleen, liver, kidneys, lungs, among others. More recently was reported in the mammary glands of bitches, although never been identified in milk. This report describes a 6-year-old female Keeshond that given birth to four puppies, three stillborn and one full-term, healthy and nursing, 20 days before biological samples collection. The bitch was serologically positive for *Leishmania* spp. in ELISA and RIFI tests and with a parasite load in bone marrow of 822,739.44 DNA copies/mL by *q*PCR. She was apparently healthy and with normal laboratory tests, except for an elevation of globulins (5.3 g/dL). Milk was collected by milking from different breasts, stored in sterile microtubes and sent for molecular tests - conventional PCR, *q*PCR and PCR-RFLP in an attempt to detect the presence of *Leishmania* spp. DNA, quantify the parasite load and characterize its species. For conventional PCR, kinetoplast DNA - kDNA, was used in the following primers: 150 - 5' (C/G)(C/G)(G/C) CC(C/A) CTA T(T/A)T TAC ACC AAC CCC 3' and 152 - 5' GGG GAG GGG CGT TCT GCG AA

3', generating a 120bp fragment. *Leishmania* spp. gDNA was detected and parasite load obtained was 683.686,87 DNA copies/µL (Probe-based qPCR targeting kDNA minicircle - Tecsa® Laboratories). The specie was characterized through PCR-RFLP by using the Internal Transcribed Spacer - ITS1 as target with an amplicon of, approximately 350bp amplified with the following primers: L5.8S: 5'- TGA TAC CAC TTA TCG CAC IT -3' and L5.8SR: 5'- AAG TGC GAT AAG TGG TA -3', digest with HaeIII restriction enzyme which allowed the identification of *L. infantum*. This is the first report of the presence of *Leishmania infantum* in milk from a lactating bitch. Further studies should be conducted to assess the importance of this finding in the epidemiology of CanL.

Keywords CANINE LEISHMANIOSIS; MILK; PCR; *Leishmania infantum*

P3-026: *Leishmania sp.* IN FEMOROTIBIOPATELAR JOINT OF A BITCH

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Leishmania sp. is an intracellular protozoan that parasitizes some mammals, including humans and dogs. Belonging to the Kinetoplastida order and Trypanosomatidae family, it has two most important evolutionary forms, amastigote which is found in vertebrate hosts and promastigote in the insect vector. *Leishmania (L.) infantum* amastigotes parasitize and infect the most diverse organs, and can cause multiple clinical manifestations, with locomotor and orthopedic alterations being the least described in the literature. The objective of this work was to report the presence of *Leishmania* sp. in femorotibiopatellar joint of a bitch domiciled in the city of Teresina, Piauí, Brazil. In August 2021, a one-year-old female French Bulldog, weighing 9 kg, was taken to a veterinary clinic for to present apathy and anorexia. On physical examination, lymphadenopathy and hyperthermia were observed. Blood count and serum biochemistry were performed, which revealed normocytic normochromic anemia, thrombocytopenia, leukopenia with absolute neutropenia and monocytopenia, and serum biochemistry showed normal values for the canine species. Abdominal ultrasound revealed hepatosplenomegaly. By means of the ELISA serological test, the diagnosis of Visceral Leishmaniasis (VL) was confirmed. Treatment with Miltefosine at a dose of 2mg/kg/day was started for 28 days; Allopurinol 10 mg/kg, every 12 hours of continuous use and Macrogard® 90 mg (immunomodulatory supplement). In November 2021, the animal began to present edema in the right femorotibiopatelar joint, causing lameness. New exams were performed and

the blood count and serum biochemistry showed no significant changes; on radiography, it was possible to observe the presence of swelling in soft tissues associated with the fourth digit without compromising bone structures, and in the parasitological examination, performed by means of aspirate of popliteal lymph nodes, bone marrow and the right femorotibiopatellar joint, the presence of of *Leishmania (L.) infantum* amastigotes. With this result, new drugs were prescribed: Condroton® 500mg, every 12 hours, for 30 days; Maxicam® 0.5mg, orally, once a day, for 5 days and Leucogen® 1mL/kg/day, for 10 days, providing a clinical improvement of the animal. In canine VL, osteoarticular changes are considered atypical, and the presence of lameness, edema, arthralgia, joint stiffness, polyarthritis and painful sensitivity to joint palpation have been described. These can be caused by the presence of the parasite and/or the deposition of immune complexes in the synovial membrane that induce inflammation and chemotactic factors that lead to joint destruction, in addition to vasculitis and increased vascular permeability, resulting in edema and even osteolysis. The diagnosis of VL through synovial fluid aspirate is a simple method that has good specificity, however it is rarely used and its sensitivity depends on the degree of parasitemia. That way, the importance of VL diagnosis by means of aspiration of synovial fluid from joints in patients who have alterations in the locomotor system, especially in endemic regions, as well as an adequate clinical evaluation that allows the identification of atypical lesions of the disease, providing an early diagnosis and a satisfactory prognosis to the animal.

Keywords AMASTIGOTE; ARTICULATE; CANINE; VISCERAL LEISHMANIASIS

P3-027: DIFFUSE CUTANEOUS LEISHMANIASIS IN BOLIVIA. A NEW CASE AND CRITICAL REVIEW

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Cutaneous and visceral leishmaniases are endemic diseases in Bolivia. Cutaneous leishmaniasis (CL) occur from the Amazonian lowlands to the sub-Andean area below 2000 m, including valleys and the Chaco region, in six out of nine Departments. While, sporadic cases of visceral leishmaniasis were reported in the Yungas of La Paz Department (1000- 2000 m), as well as to the eastern lowlands of Santa Cruz Department (Le Pont et al., 1992). *Leishmania braziliensis* is the most frequent species responsible of cutaneous and mucosal leishmaniasis (ML). *L. amazonensis* is the second species involved in CL and may produce exceptionally, the particular clinical form named diffuse cutaneous leishmaniasis (DCL). The first case of DCL of the world, was identified in 1946 in Bolivia (Prado Barrientos, 1948), without identification of the *Leishmania* species. A second case of DCL was published by Valda in 1980; nevertheless, the poor quality of the microphotography published, showing "apparent amastigotes" in the skin patient´s samples was not compatible with this aetiological diagnosis, according our large experience; additionally, the lesions of the patient were more compatible with Hansen's disease rather CDL. Another case was discovered in 1993 (Martinez et al, 2001), corresponding to a little Aymara girl co-infected by *L. amazonensis* and *Leishmania infantum*. This was the first well-known co-infection due two *Leishmania* species in the same lesions and the first-one with aetiological confirmation from Bolivia. We

present a case of DCL in Bolivia, corresponding to an adult male patient with diffuse nodular, infiltrative and non-ulcerated lesions (in the face, thorax and extremities); cicatrized cheloid-like lesions in the hands, and verrucous-like lesions in the foot-fingers. The patient, was born and living in a rural region in the tropical humid Amazon rain forest, at the northern Department of Pando near the border with Brazil. The disease began progressively more 10 years ago, with periods of spontaneous exacerbations and minimal remissions. The Giemsa-stained smears showed abundant free amastigotes, as well as many vacuolated histiocytes containing numerous parasites. The cultivation in NNN media showed successful adaptation and growth of promastigotes. The Montenegro skin test was negative (0 mm) after 48 and 72 h. Inoculated hamsters exhibited granulomas at the inoculation site that evolved to voluminous and serious lesions showing tendency to ulceration, contained abundant intracellular and extracellular amastigotes, vacuolated histiocytes, similar to observation from patient's samples. The clinical characteristics, the abundance of parasites and vacuolated histiocytes in the lesions, and the negative skin test, confirms the diagnosis of DCL. The morphological characteristic of the amastigotes and promastigotes, the behaviour in cultures and inoculated hamsters are compatible with *L. amazonensis*. The definitive specific diagnosis is in course. The patient received three complete treatment series for 30 days with pentavalent antimonial (meglumine) with partial remission and one with liposomal amphotericin B, without results. This is the third case of CDL in Bolivia supported with clinical and parasitological arguments, unfortunately, the patient remains in their community without changes, in contrast, their general condition is good. We are exploring possibilities to treat this exceptional case.

Keywords LEISHMANIASIS; DIFFUSE CUTANEOUS LEISHMANIASIS; *Leishmania amazonensis*; BOLIVIA

P3-028: *Leishmania brasiliensis* DETECTION IN BLOOD PERIPHERAL EXAMINATION OF PACIENT WITH HIV: CASE REPORT

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Leishmaniasis is an infectious disease, caused by protozoa from *Leishmania* genus and vector transmission. It has a wide clinical range, which can be divided into 3 large groups: Visceral Leishmaniasis, Mucocutaneous Leishmaniasis and Cutaneous Leishmaniasis, the last one can occur with a few or numerous lesions, depending on the host immune response and the parasite species. In this condition, it can be classed as disseminated form when these multiple lesions affect many body segments. The diagnosis depends on parasite detection in lesions that often have a scarce causative agents. Thus, the detection in blood peripheral examination can strongly support the diagnosis. It collected data from the medical records of the admitted patient in a reference service in infectiology at the Mato Grosso state. A 38-year-old man, who has had HIV since 2009, was poorly engaged in the treatment, he was admitted to the reference service to infectious diseases in Cuiabá-Brazil. Patient with Liposomal Amphotericin B cycle by the previous diagnosis of Leishmaniasis Tegumentary with lesions resolutions in August 2019. Hospitalized in February 2020, with HIV viral load: undetectable and CD4: 18 cell/mm^3 (August 2019), with daily fever, arthralgia, strength reduction in the upper and lower limbs, paraesthesia, eye pain associated left amaurosis for 10 months, and reduction of the right visual acuity. Physical examination: hyperchromic macules, spread in the body associated with nodules, hyperemic and painful to palpation, bilateral inguinal lymphadenopathy. Rhinoscopy: left nasal septum with roughness and granulation and right blood crust. Direct parasitology of nasal mucosa lesions and left inguinal lymph node with shapes of Leishmania amastigotes,

treated with 30mg/kg of Liposomal Amphotericin B with good clinical response to the therapy. In May 2021, the patient had a neurotuberculosis diagnosis (CD4: 46 cell/mm3) and returns to the daily fever arthralgia, odynophagia, and new nodule lesions throughout the body. New skin biopsies confirm the *Leishmania sp* by direct exam, culture medium and histopathological. due to the systemic scenario, it was chosen the blood peripheral sample to the examination of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in a research lab, identified *Leishmania braziliensis*. The patient was treated with many combinations, among them, Liposomal Amphotericin B, miltefosine, and pentamidine, which resulted in a good clinical response.The significance of this case is to demonstrate that patients who develop disseminated integumentary forms can eventually have these multiple lesions together with the systemic features and parasite dissemination so that it is possible to bring back the leishmania in the blood peripheral exams. Considering that the Leishmaniasis parasitological diagnosis is tough due to the scarcity of the etiologic agent, this ascertainment can be useful. There are just a few records of this condition in the available literature.

Keywords LEISHMANIOSIS; *Leishmania*; HIV; DISSEMINATED

P3-029: ANTIMONY (Sb^{III}) AND CELECOXIB COMBINATION AS AN ALTERNATIVE FOR CUTANEOUS LEISHMANIASIS

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The combined therapy using one antileishmanial compound plus one anti-inflammatory could be an interesting strategy for cutaneous leishmaniasis (CL) treatment. The aim of this study was to evaluate the antileishmanial activity of a combination of trivalent antimony (potassium antimony tartrate, Sb^{III}) with celecoxib in experimental CL. The potency of one drug (Sb^{III} or celecoxib) and combinations was evaluated against promastigotes, axenic amastigotes, intracellular amastigotes of *L. (V.) panamensis* and *L. (V.) braziliensis*. A topical oil-formulation containing a combination was prepared, characterized and evaluated in CL-infected BALB/c mice for 28 days with a following-up of 30 days. An irritation/corrosion test was performed for 21 days. Skin and lesion histopathological analyzes were performed. The activity of Sb^{III} was higher in axenic amastigotes (IC_{50} 18.4 to 23.1) than promastigotes (IC_{50} 38.6 to 43.8) and intracellular amastigotes (66.9 to 75.4), while for celecoxib it was higher in promastigotes (IC_{50} 35.1 to 43.7). The interactions Sb^{III}-celecoxib was indifferent/additive (mean ∑FICs 1.22 to 1.51). The selective index for both compounds was <1. The prepared ointment-formulation was stable at room temperature up to 40 days. Thirty-days after treatment, 1% Sb^{III} plus 0.75% celecoxib induced a 90 to 100% of lesion reduction, complete re-epithelialization, and no reactivation in 37.5% mice; however, necrosis and erythema were observed. One-drug treatment was not effective. No signs of dermal irritation or corrosion were observed in healthy skin after treatment Low acanthosis and hyperkeratosis at the epidermis and abundant mixed inflammatory infiltrate with amastigotes were observed except in 18.8% of the samples that were parasite free. Both, individual and combined Sb^{III} + celecoxib was

active against *Leishmania* however, no synergistic drug interaction was observed (FIC <0.5) *in vitro.* In mice, combined but not one-drug treatment showed some effectivity (37.5%). A better effective/non-toxic dose still needs to be found.

Keywords TRIVALENT ANTIMONY (SB^{III}); CELECOXIB; COMBINED THERAPY; DRUG INTERACTIONS; CUTANEOUS LEISHMANIASIS

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P3-030: OTHER TOPICAL ALTERNATIVES FOR THE MANAGEMENT OF CUTANEOUS LEISHMANIASIS, APARTADÓ, COLOMBIA

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Cutaneous leishmaniasis is endemic and represents a serious public health problem in the Urabá region, Colombia, registering an incidence rate of 57.5 cases/100,000 inhabitants in 2020. Pentavalent antimonials (meglumine antimoniate) are the first-line treatment drugs for cutaneous Leishmaniasis, at doses of 20 mg / kg / day, intramuscularly or intravenously, in a single daily dose, generating side effects, such as nephrotoxicity, hepatotoxicity, acute and pancreatitis alterations at the cardiac level, among others. PAHO recommends minimally invasive therapeutic options for the management of uncomplicated cutaneous leishmaniasis. The objective of this study was to describe the response to a new topical treatment in patients with cutaneous leishmaniasis, treated at the Colombian Institute of Tropical Medicine, Apartadó. This is an observational and retrospective descriptive study based on a review of the medical records of 69 patients treated with Alyeyuba® emulsion and lotion (*Caesalpinia spinosa* extract), applied 3 times a day for 30-60 days. 57% (39/69) of the patients treated with Alyeyuba® met the criteria for cure with a satisfactory clinical response, 21,5% presented therapeutic failure and the remaining and 21,5% were lost to follow-up. A greater cure was observed in women (85.7%), especially housewives (100%), than in men, as well as in patients without bacterial infections (60.9%), without the use of previous alternative treatments (59.3%) and in injuries located in the head (75%). A statistically significant difference was found between the time of evolution of the lesions and the

response to treatment, with 75% of the patients who were cured presenting lesions of more than 100 days. In addition, it should be noted that there is a trend that the longer the treatment time, the higher the cure rate, since 75% of the patients with a satisfactory response were treated for more than 30 days. In the present study, the Alyeyuba® treatment was satisfactory in 57% of the patients with cutaneous leishmaniasis, and it was possible to determine that the longer the time of evolution of the lesions, the higher the cure rate. Although this percentage is lower than that of the drugs of first choice, it must be considered that topical treatment does not seem to have side effects, is easy to apply and is inexpensive.

Keywords CUTANEOUS LEISHMANIASIS; TOPICAL TREATMENT; ALYEYUBA®; *Caesalpinia spinose*

P3-031: IMPACT OF THE Leishmania RNA Virus 1 ON THE SUSCEPTIBILITY OF *Leishmania* (*Viannia*) *braziliensis* STRAINS TO TRIVALENT ANTIMONY

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Tegumentary leishmaniasis is a dermotropic disease caused by the etiologic agent *Leishmania*. Annually, between 600,000 to 1 million new cases of this disease are registered in 88 countries distributed in tropical and subtropical areas of the world. However, there are reports of therapeutic failure with these drugs in some parts of the world. One of the possible associated factors is the presence of the viral endosymbiont Leishmania RNA Virus 1 (LRV1) in parasites of the subgenus *Leishmania* (*Viannia*). In this context, the present work analyzed the LRV1 positive and LRV1 negative groups for the *In vitro* susceptibility to the trivalent antimony compound, the active form of the pentavalent antimony prodrug. For this study, 20 strains of *Leishmania* (*Viannia*) *braziliensis* isolated from patients diagnosed with TL at the Centro de Medicina Tropical de Rondônia (CEMETRON) were

selected, and 12 of these strains are positive for the viral endosymbiont. The growth curve of all strains was evaluated to determine the logarithmic phase and synchronize the parasites' growth phase for the experiments. These strains showed logarithmic phases between 72h and 96h of growth under the same culture conditions. These strains showed logarithmic phases between 72h and 96h of growth under the same culture conditions. From this result, the quantification of the viral load of positive strains for LRV1 and the *In vitro* susceptibility experiments to the trivalent antimony were carried out. Among the 12 strains positive for LRV1, it was possible to quantify the viral load of nine strains (75%). For three strains (25%), it was not possible to determine the load as they were not detected within the quantification limit of the standard curve. The viral load of the nine LRV1 positive strains ranged from 29.112,80 copies/reaction to 1.867.804,07 copies/reaction. In parallel with this assay, the *In vitro* susceptibility of the strains to the trivalent antimony compound was evaluated. The results showed two groups of susceptibility to the trivalent antimony compound, the (i) susceptible (N= 8) and (ii) less susceptible (N= 12), when considering the patterns of parasite density in the smoothing curves, while in the evaluation of 50% growth inhibition (IC_{50}). In the activity index (AI), all strains were less susceptible than the susceptible strain IOCL566 used as reference. Viral load and the presence of the *LRV*1 endosymbiont were related to *In vitro* susceptibility to the trivalent antimony. From the results obtained, it is concluded that there is an intraspecific variability in the *In vitro* susceptibility profile to the trivalent antimony compound. Furthermore, this variability is not related to the presence and viral load of the *LRV*1 virus in the parasites in the axenic system since positive and negative strains for the endosymbionts are less susceptible to the trivalent antimony drug. Assumptions from those results may be relevant, but it is essencial to go further in a more complex system with parasite-host interactions before taking any statements on the effects of *LRV*1 presence.

Keywords TEGUMENTARY LEISHMANIASIS; *In vitro*; IC_{50}; VIRAL LOAD

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P3-033: MOLECULAR EVALUATION OF THE RESISTANCE TO THE MEGLUMIN ANTIMONIATO (GLUCANTIME®) IN LEISHMANIA ISOLATES FROM PATIENTS WITH THERAPEUTIC FAILURE

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The leishmaniasis is a tropical disease of vector transmission caused by parasite of the Leishmania genera, which is widely extended in the whole world, especially in tropical regions and persons with low income (Handler y col, 2015). The therapeutic options for the treatment of this disease include conventional drugs such as pentavalent antimonial (SbV), Amphotericin B, Miltefosina, Pentamidina y Paramomicina (De Guglielmo y col, 2018). However the acquired resistance to the Glucantime (Berg y col, 2013) has been increasing with the pass of the years (Lira y col, 1999), this has obligated to the authorities to the application of new therapeutic alternatives. The resistance to antimonial will be evaluated using phenotypic, proteomic or genomic analysis (Jeddi y col, 2011). The most common is the genomic analysis using the DNA restriction fragments **.** The main objective of this work was the identification of possible genetic markers related to drug resistance in cases of localized cutaneous leishmaniasis using a comparative analysis of the genetic profile of resistant and non-resistant strains to Glucantime. For this proposal the induction of resistance *in vivo* and *in vitro* to the drug was performed using the therapeutic dose of glucantime with an international reference strain of *L. (V.) braziliensis* and compared with samples from patients with or without therapeutic failure. Digestion with restriction enzymes (HindIII, PstI, MspI) and 1% agarose gel electrophoresis were performed, using manual comparison and descriptive statistics for the analysis. DNA fragments of greater intensity were evident, with a molecular weight between 2,0 and 1,5 kb in one of the patients without clinical cure (HindIII), and two distinctive DNA fragments of 0,65 kb and 0,5 kb (MspI) in the isolated with

experimental induction of resistance. The majority of the patients came from Miranda state and it is the probably site of infection (57,14% and 71,42%, respectively), and a minority (28,58%) had a therapeutic failure. It was possible to identify variations in the restriction profiles of experimentally resistant parasites and that from patients with therapeutic failure. A unique DNA fragment of 0.6 Kb was obtained after digestion of the DNA obtained from a patient with no respond to the treatment with glucantime digested with MspI restriction enzyme. This will be a molecular marker to evaluate the resistance to the treatment with pentavalent antimonial. In addition, a new method for the induction of drug resistance under *in vivo* and *in vitro* conditions was proposed.

Keywords LEISHMANIASIS; TREATMENT; RESISTANCE; MOLECULAR MARKERS

P3-034: LIPOSOMAL AMPHOTERICIN B FOR DISSEMINATED CUTANEOUS LEISHMANIASIS IN A PEDIATRIC PATIENT WITH DOWN SYNDROME. A CASE REPORT.

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Cutaneous Leishmaniasis can be classified as Localized, Diffuse, or Disseminated disease depending on the clinical presentation and course. These variations are due to the interaction of multiple factors, mainly the *Leishmania* species, and the immunological status of the patient. In this report, we present the case of a child with Down syndrome who developed a disseminated form through a long period of time. This is a 5 year old female patient, coming from Quillabamba province in Cusco Region, Peru. The parents describe the development of small papules in the child´s face at one year of age. These lesions grew very slowly, and after a year, these papules ulcerated in the right malar and mandibular areas of her face. At that time, cutaneous leishmaniasis was diagnosed and treated with amphotericin B deoxycholate 259mg total over 37 days. One year later, the patient was hospitalized with multiple papular and ulcerative lesions in the face, buttocks, legs, and arms, plus respiratory distress and laryngeal stridor. The patient (weight 12 Kg) received amphotericin B deoxycholate for a cumulated total of 952mg over 5 months plus sodium stibogluconate for 25 days, plus topical imiquimod and miltefosine 30 mg/day for two months. Initial response was poor and lesions never healed fully, nevertheless the patient was discharge after completing treatment and lost follow-up. Two years later, the patient was hospitalized again because the never healed lesions were growing in size. At physical exam, there were numerous cutaneous raised ulcerated lesions distributed in legs, arms, buttocks, and

face with diameters from 1 to 8 cm, plus presence of small 0.5 cm erosions in the oral mucosa. Lesions smear and pathology examination revealed abundant amastigotes. Montenegro test was positive and serology showed 1/160 titers for anti-*Leishmania* IgG. Using PCR-RFLP, the Hsp70 gene was amplified, typifying the parasite as *Leishmania (Viannia) braziliensis.* Celular Immunological status was evaluated using flow cytometry finding low levels of CD3/CD4 lymphocytes, T-reg lymphocytes, and T Helper CD4. Final Diagnosis was Disseminated Cutaneous leishmaniasis (DL) and the patient was treated with Liposomal amphotericin B IV 3mg/kg/day every 5-6 days, for a total of 15 doses. Patient experienced a marked recovery after the 11th dose, and almost full recovery after finishing treatment. This case illustrates how *Leishmania (Viannia) braziliensis* can produce a very aggressive form of the disease in patients with some immunological compromise. In this case, a child with Down syndrome that affects the cellular immunological response, who was also treated with corticosteroids for a prolonged period of time. It is remarkable that the patient has presented cutaneous lesions during four years without healing fully at any interval of time, and even presenting mucosal lesions intermittently despite receiving full course of antimonials and amphotericin B deoxycholate. In cases of disease with a protracted course and non-healing lesions, we consider it is mandatory to type the *Leishmania* species, and evaluate the immunological status of the patient. Finally, it is important to highlight, that after four years, the patient finally experienced an almost completely recovery after receiving Liposomal amphotericin B. This form of amphotericin allows for higher doses during prolonged periods of time; and it is suitable to treat more aggressive or persistent forms of the disease.

Keywords DOWN SYNDROME; CUTANEOUS LEISHMANIASES; LIPOSOMAL AMPHOTERICIN B; *Leishmania braziliensis*; PERU

P3-036: EVALUATION OF SCARS AND TREATMENT PREFERENCES IN SUBJECTS WITH CUTANEOUS LEISHMANIASIS TREATED WITH PENTAVALENT ANTIMONIALS, THERMOTHERAPY OR THERMOTHERAPY IN COMBINATION WITH MILTEFOSINE, A MULTICENTER STUDY COLOMBIA AND PERU

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Pentavalent antimonials continue to be the first treatment option for cutaneous leishmaniasis in Latin America despite their known adverse events; lately, the need has arisen to seek effective therapeutic alternatives, with shorter treatment schedules and better safety profiles. Currently, the Patient Reported Outcomes (PRO) initiative has been developing and finds an important source of information in the opinions and thoughts of people with specific health conditions. In the case of cutaneous leishmaniasis, the perspective of patients regarding therapeutic options and treatment presentations has been little evaluated, so this study aimed to compare the preferences of patients with cutaneous leishmaniasis who received treatment with pentavalent antimonials, thermotherapy or combination therapy plus Miltefosine. A psychometric and prospective evaluation study was carried out, nested to the clinical trial code NCT02687971[1], in addition, volunteer patients treated with pentavalent antimonials were included. An instrument for assessing treatment options was used with 8 cards

containing different systemic and local treatment alternatives and treatment combinations, where the participant chose, in order of preference, the three options that he considered the best for the therapeutic management of the disease. This instrument was evaluated before the start of treatment and at the end of it. A total of 75 volunteers participated in the study, distributed in 3 treatment groups: glucantime (N=10), thermotherapy (N=32) and a combined treatment[2] (N=33). All volunteers were comparable in sex, age, race, and occupation (p-value >0.05) At the beginning of treatment in the group that received Glucantime, the most chosen option was the systemic alternatives (intravenous (30%) and oral (30%)) and in the evaluation at the end of treatment the preferred option was the application of medication in the lesion 3 times a day for 1 month (56%). In the group of participants who received thermotherapy as monotherapy or in combination with miltefosine, the therapeutic alternatives chosen at the beginning and at the end of treatment excluded systemic options. The study population considers that local therapeutic alternatives headed by topical treatments (thermotherapy and cream application) would be the best treatment options for the management of cutaneous leishmaniasis, which prompts us to continue the search for other different therapeutic options. to systemic options.

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P3-037: EVALUATION OF SCARS IN PATIENTS WITH CUTANEOUS LEISHMANIASIS TREATED WITH DIFFERENT SYSTEMIC AND LOCAL ALTERNATIVES - VANCOUVER SCAR SCALE ASSESSMENT

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Leishmaniasis is a parasitic disease that presents a great clinical polymorphism, being Cutaneous Leishmaniasis (CL) the most prevalent form, with the appearance of frequent lesions in exposed anatomical areas such as the face and extremities. Although the disease is not fatal, many who have suffered from it have prominent scars and even deforming their quality of life. There are systemic and local alternatives for the treatment of LC, such as: pentavalent antimonials, miltefosine, pentamidine, paramomycin, amphotericin B, thermotherapy, cryotherapy, among others; none have demonstrated universal efficacy, some have safety drawbacks and there is no quality scientific evidence to support the use of each type of treatment according to the characteristics of the populations.The objective of this study was to evaluate, by applying the Vancouver scar scale, the type of scar in a study group with patients diagnosed with LC who received different therapeutic alternatives, such as: pentavalent antimonials, thermotherapy and combined therapy. of miltefosine plus thermotherapy. Prospective study, nested in the clinical trial NCT02687971.The volunteers' scars were

evaluated at 45, 90 and 180 days post treatment, in order to evaluate their evolution and final result, using the Vancouver Scar Assessment Scale as a tool, which evaluates pigmentation, vascularity, flexibility and height of the scars. The evaluator was blinded to the treatment received by the participant. A total of 76 patients (53 patients from Colombia and 23 patients from Peru) took part in the study. Of these 63 were men. Participant ages ranged from 18 to 66 years, with a median of 39 years. Regarding treatment, 33 individuals received thermotherapy, 33 received a combined scheme of thermotherapy and miltefosine, and 10 of these patients received glucantime. The evaluation of healing tendency found that 57 patients (75%) had a normal-appearing scar (26 treated with thermotherapy, 26 patients in combination with thermotherapy with miltefosine and 5 with glucantime); 12 patients (16%) had a hypertrophic scar (5 treated with thermotherapy + miltefosine, 4 with thermotherapy and 3 with glucantime), another 6 participants (8%) had an atrophic scar: (3 treated with Glucantime, 2 with thermotherapy and 1 combined treatment); 1 volunteer (1%) presented a keloid scar who received thermotherapy + miltefosine. LC is a condition that can become stigmatizing due to the type of scars it produces, however, and according to the results found, these are not related to the type of treatment used, be it local or systemic; This favors the use of other therapeutic alternatives, preferably non-invasive. Studies with a larger sample size are needed to validate these results.

Keywords THERAPY; TREATMENT; HEALING; CICATRIZATION

P3-037.1: METHODOLOGICAL VARIATION IN DESIGN AND CONDUCT OF DRUG EFFICACY STUDIES IN VISCERAL LEISHMANIASIS: A SYSTEMATIC REVIEW OF PUBLISHED LITERATURE

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Variations in methodology adopted in different aspects of study design and conduct has been relatively under-researched in visceral leishmaniasis (VL). A systematic review of all published therapeutic-based studies in VL was carried out to characterise the methodological aspect of clinical studies. This review collates data from published studies conducted between 1980 and 2021, indexed in the Infectious Diseases Data Observatory (IDDO) VL living systematic review of clinical studies. The data extracted from the studies in the IDDO VL library included: inclusion & exclusion criteria, case definition used for patient screening, tissue aspirate(s) used for confirmation of VL at enrolment and during follow-up, early and late therapeutic outcomes adopted, and time-point of outcome evaluations. All studies 160 trials indexed in IDDO VL library were included. Inclusion/exclusion used for patient enrolment was not reported in 36 (22.5%) studies. Patients with at least one co-morbidity were excluded in 92 (57.5%) studies with the following co-infections being excluded: HIV (n=77 studies), hepatic disorders (n=57), tuberculosis (n=54), renal disorders (n=51), and cardiac disorders (n=48). Case definition for patient screening was defined solely using compatible clinical diagnosis in 27 (16.2%) studies, parasitological confirmation in 37 (23.1%), a combination of compatible clinical diagnosis and/or parasitological/serological methods in 81 (50.6%), and was unclear

in 57 (9.4%). After initial screening, VL confirmation required examination of tissue aspirates from spleen in 54 (35.5%) studies, bone marrow in 22 (14.5%), either bone marrow and/or spleen in 53 (34.9%), combination of one or more of the above in 19 (12.5%), and the sample was unclear in 4 (2.6%). Commonly adopted Initial therapeutic endpoints included initial cure (n=53 studies), clinical cure (n=52), parasitological cure (n=50), and unresponsiveness (n=18 studies). Test of cure was carried out after < 15 days of post-treatment in 7 (4.4%) studies, between 15–30 days in 110 (68.8%), between 31 to 70 days in 18 (11.3%), between 15-70 days in 9 (5.0%), and was unclear in 20 (12.5%). Relapse was defined based on clinical suspicion in 3 (1.9%) studies, parasitic demonstration in 22 (13.8%) studies, a combination of clinical and/or parasitological/serological methods in 36 (22.5%) studies, and the methodology was not defined in 99 (61.9%) studies. This review highlights substantial methodological variations in definitions adopted for patient screening, disease diagnosis and therapeutic outcomes used, and incomplete reporting of several methodological aspects in published VL studies, emphasizing the need for harmonisation of endpoints, definition and trial practices to help mitigate methodological heterogeneity across studies. Dissemination and use of recently developed CDISC compatible CRF for VL trials would help addressing this challenge.

Keywords VISCERAL LEISHMANIASIS, NEGLECTED TROPICAL DISEASES, DESIGN, REVIEW, METHODOLOGY

P4-011: BACTERIAL CELLULOSE BIOCURATIVES FOR THE TOPICAL TREATMENT OF CUTANEOUS LEISHMANIASIS

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In Brazil, cutaneous leishmaniasis (CL) is mainly caused by *Leishmania braziliensis*. Pentavalent antimonials (Sb^v) remain the first-line drug on treatment for CL despite the limitations regarding toxicity and increasing reports of therapeutic failure. Therefore, the search for alternative options for treatment that are safe, efficient and of easy application remains necessary. We have show that DETC, a SOD1 inhibitior, in association with a bacterial cellulose (BC) biocurative, reduced parasite burden and inhibited lesion development in a pre clinical model of CL caused by *L. braziliensis*. We thus hypothesized that BC biocuratives in association with DETC (BC-DETC) could act in conjuction with pentavalent antimonials to reduce the burden of disease in CL patients. To this end, we performed physicochemical characterization of BC-DETC employing scanning electron microscopy (SEM) and x-ray diffraction (XRD). In addition, we performed an *in vitro* release assay by spectrophotometry and evaluated the stability of DETC onto BC by spectrophotometry and thermogravimetry. SEM images of BC-DETC showed DETC aggregates across the entire surface. The absence of crystallographic peaks, seen by XRD analysis, indicated that DETC was succesfully incorporated onto BC biocuratives. In vitro release experiments showed a accumulative mass release of 22% and 14%, at 5 minutes and 24 hours, respectively, indicating possible degradation of DETC. Thermogravimetry analysis complemented our findings that strongly indicating that DETC is not stable when incorporated onto BC. Despite our

results showing that DETC is short lived when incorporated onto BC, as suggested by degradation experiments, we performed an initial a pilot, proof-of-concept trial, to evaluate the efficacy of topical application of BC in CL patients. A total of 20 patients were randomized in two groups assigned to receive either parenteral Sb^v alone or parenteral Sb^v plus topically applied BC bio-curatives. CL patients treated with Sb^v + topical BC bio-curatives had a significantly higher cure rate at 60 days post initiation of treatment compared to CL patients treated with Sb^v alone (P=0.01). At day 90 post initiation of treatment, cure rate was similar in the two groups as was overall healing time. Adverse effects or local reactions to topical BC application were not observed. This pilot trial shows that the potential use of a combined therapy consisting of topical BC bio-curatives and parenteral Sb^v in favoring healing of CL lesions caused by *L. braziliensis*, at an early time point.

Keywords CHEMOTHERAPY; TOPICAL TREATMENT; BIODRESSING

P4-012: DEVELOPMENT OF MICROPARTICULATED IMPLANTS BY SPRAY DRYING FOR SUSTAINED RELEASE OF ANTILEISHMANIAL DRUGS

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Local cutaneous leishmaniasis (LCL) therapy is based on multiple injections with drugs that can cause severe systemic toxicity. Pain, unwellness, and the required frequent visits to distant hospitals are obstacles for treatment completion. Since topical creams have not demonstrated adequate drug absorption and effectiveness, we envisaged to develop novel drug formulations that allow a single local injection to be effective. For that, using spray drying technique with a three-fluid nozzle set, which allows a shell-core structure, we produced biodegradable PLGA (poly(lactide-co-glycolide acid) microparticles blended with PLA or PVP containing amphotericin B (AmB) in the core, as a prototype formulation for the treatment of LCL in a sustained drug release fashion. The fabrication process showed 60-70% yield; average particle size of 13,5 µm; zeta potential of -12,6 mV, and >70% drug incorporation rate. MEV images showed spherical and rough particle topology. *In vitro* drug release kinetics showed that PLGA/AmB microparticles promoted much slower release than free AmB within 48 hours. Cytotoxicity studies using murine BMDM cells showed that PLGA/AmB and PLGA microparticles were non-toxic to mammalian cells. Histopathological studies in mice showed that PLGA/AmB particles injected s.c. were effectively taken up by dermal macrophages. BALB/c mice infected in the ear pina with *Leishmania amazonensis*-GFP were given a single s.c. injection with PLGA/AmB or free AmB in deoxycholate (Anforicin®). PLGA/AmB-treated lesions significantly controlled lesion growth and

parasite burden as compared with free AmB. This study shows the effectiveness of spray drying fabrication of microparticulated PLGA/AmB implants in the treatment of local cutaneous leishmaniasis, and their potential applicability with newly discovered antileishmanial drugs.

Keywords CUTANEOUS LEISHMANIASIS; CHEMOTHERAPY; AMPHOTERICIN B; POLYMERIC MICROPARTICLE; SPRAY DRYING

Financing Capes; Vale do Rio Doce

P4-013: PHARMACOMODULATION OF ORIGINAL AMIDOXIMES AS ANTILEISHMANIAL AGENTS

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Leishmaniasis, as a neglected disease, requires safer and less expensive new oral treatments. In view of this general interest, our project concerns the synthesis of amidoxime derivatives presenting a 2,3-dihydrofuran heterocyclic scaffold that could offer a new option for the treatment of *Leishmania* disease. Pharmacomodulation in a ligand-based approach allows to explore the influence of substituents on antileishmanial activity and to focus on monoamidoximes scaffolds. In this context, 2,3-dihydrofuran scaffold of original structure bearing the amidoxime group were synthetized by a three-step procedure with different strategies: manganese(III) acetate radical oxidative cyclization by microwave irradiation, transition metal catalyzed coupling reactions, amide bond formation and β-ketosulfone formation leading to heterocyclic derivatives. The reagent was selected mainly with the aim of improving the activity without significantly increasing toxicity and improving the physicochemical properties such as solubility. Amidoximes derivatives synthetized were subjected to *in vitro* biological assessment. The cytotoxicity evaluation on murine peritoneal macrophages and the antileishmanial activity on *Leishmania amazonensis* (MHOM/BR/77/LTB0016) were thus evaluated with antipromastigote and antiamastigote assays performed at the Oswaldo Cruz Foundation – FIOCRUZ, giving the 50% cytotoxic concentration (CC50) and the 50% inhibitory concentration (IC50). The selectivity Index was

calculated according to the formula: SI = (Murine macrophages CC50) / (*L. amazonensis* amastigotes IC50). Two principal HITs are our current reference, the 4-(5-benzyl-3-(4-fluorophenylsulfonyl)-5-methyl-4,5-dihydrofuran-2-yl)-*N*′-hydroxy-benzimidamide (*L. amazonensis* promastigotes IC50 : 5.4 ± 1.0 µM, *L. amazonensis* amastigotes IC50 : 7.9 ± 1.1 µM; CC50 : 102.0 ± 2.8 µM), and its methylate derivative in the benzyl group (*L. amazonensis* promastigotes IC50 : 5.6 ± 0.9 µM, *L. amazonensis* amastigotes IC50 : 6.7 ± 1.2 µM; CC50 : 111.5 ± 7.3 µM) with a SI of 12.9 and 16.6 respectively. Both with a better selectivity index than pentamidine which is 4.5. (Ref. pentamidine IC50 : 1.9 ± 0.12 and CC50 : 8.5 ± 1.3 µM). From our ongoing work, we have previously reported that the presence of the dihydrofurane and amidoxime groups is necessary for antiparasitic effect, and the influence of benzyl group substitution has been demonstrated as well. On the other hand, mechanistic studies are desirable to elucidate the pharmacological mechanism of amidoximes and thus understand the observed activity. In conclusion, amidoxime derivatives presenting a 2,3-dihydrofuran heterocyclic scaffold are promising candidates for further biological evaluation. Moreover, the developed synthetic pathway allows a broad modulation towards improved physicochemical properties and activity without increasing significantly the toxicity.

Keywords MN(OAC)3; AMIDOXIMES; DIHYDROFURAN; RADICAL CYCLIZATIONS; *Leishmania*

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P4-014:STEROL REMODELING BY PHARMACOLOGICAL ACTION AND POTENTIAL THERAPEUTIC TARGETS BY THE SERINE PROTEASE PATHWAY USING A SUBTILISIN INHIBITOR

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Subtilisins (SUB) found in all organisms, are enzymes important in the post-translational steps of protein processing. Studies has been described that SUB are essential to *Leishmania*. Transcription factors such SREBP have been established as lipid synthesis transcription factors in mammalian and fungi, especially for cholesterol and fatty acid synthesis, and its factor is regulated by SUB. In *Leishmania s*p, no transcription factor was already demonstrated. The ergosterol biosynthesis pathway has been exploited as a pharmacological target. Trypanosomatids produce ergosterol and other sterols; sterols are methylated by SMT in one of the final steps of the sterol biosynthesis pathway (SBP) but this reaction does not occur in mammalian cells due to the absence of SMT. Although, available information about the mechanisms of the regulation and remodeling of sterol-related genes is scarce. In this context, we investigated compensatory mechanisms of the SBP using an inhibitor of HMG-CoA and by developing drug-resistant parasites to evaluate (sterol remodeling, cross-resistance and gene expression)as well as we evaluated potential therapeutic targets mediated by lipid way and exploring the localization and the SUB play role on *L. amazonensis*. Simvastatin-resistant *L. amazonensis* parasites (LaSimR)underwent reprogramming of sterol metabolism manifested as an increase in cholestane- and stigmastane-based sterols and a decrease in ergostane-based sterols. The levels of SMT, sterol C14-α-demethylase, and SUB were increased in LaSimR. LaSimR was cross-resistance to ketoconazole (C14DMi)and remained sensitive to terbinafine. Sensitivity of the LaSimR to other antileishmanial drugs unrelated to the SBP, such as trivalent antimony and pentamidine, was similar to that of the WT; LaSimR was cross-resistant to miltefosine, serine protease inhibitor(SPi)TPCK, subtilisin-specific inhibitor PF-429242, and tunicamycin. *Leishmania* proved to be more sensitive to SPi, thus we chose to carry out more tests using just PF-429242, which is an inhibitor of human S1P and in addition to already having tests demonstrating its effect on some microorganisms. Using catalytic domain antibody to SUB we performed TEM and FACS, which demonstrated SUB has broad localization throughout the cytoplasm and

membrane of promastigote form with foci in the flagellar pocket. In silico, the similarity between SUB of different *Leishmania* species and that of the human was determined and based on molecular docking, we evaluated the interaction capacity of a SPI against both life cycle forms of *Leishmania*. PF-429242 significantly inhibited the growth of promastigotes of four different strains (IC50= 3.07; 0.83; 2.02 and 5.83 µM against LTB0016, PH8, Josefa, and LV78 strains) whilst having low toxicity in the host macrophages(170.30µM). We detected by FACS, using a catalytic domain ab from SUB that there is a higher expression of SUB in amastigote; however, the PF-429242 had a low effect against this intracellular form with an IC50 of>100µM for intracellular amastigotes for the LV78 strain as axenic amastigotes (94.12µM). In conclusion, even though PF-429242 does not affect the intracellular forms, this drug will serve as a tool to explore pharmacological and potentially leishmanicidal targets. Additionally, findings on the regulation of the sterol pathway can support the development of drugs and protease inhibitors targeting this route in parasites.

Keywords SUBTILISIN; SERINE PROTEASE; STEROL PATHWAY; *Leishmania amazonensis*; PHARMACOLOGICAL TARGET

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P4-015: NOVEL METHODS TO QUANTIFY MILTEFOSINE, PAROMOMYCIN AND AMPHOTERICIN B IN SKIN BIOPSIES FROM POST-KALA-AZAR DERMAL LEISHMANIASIS PATIENTS

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Miltefosine [1], amphotericin B and paromomycin [2] are all approved drugs for the treatment of various clinical presentations of the neglected parasitic disease leishmaniasis. In cutaneous leishmaniasis and post-kala-azar dermal leishmaniasis (PKDL), *Leishmania* parasites reside and multiply in the dermis of the skin. These treatment options are currently studied in combination therapy trials for the treatment of these dermal leishmaniases. There is an urgent need for accurate assays to determine miltefosine, amphotericin B and paromomycin concentrations in human skin tissue, to assess target site pharmacokinetics of these antileishmanial drugs to enable further optimization of the dosing regimens. To date, no bioanalytical assay was available to assess human skin concentrations of these antileishmanial drugs. We here describe the development and validation of sensitive and accurate methods to homogenize human skin tissue and quantify miltefosine, amphotericin B and paromomycin in 4-mm human skin biopsies utilizing high-performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Quantification of pharmaceutical compounds in skin tissue is challenging because of low expected concentrations, small typical sample volumes, and hard nature of the skin structure itself [3]. Given that the true analyte recovery from skin tissue is difficult to assess, the extent of homogenization plays a crucial role in the quantification. We developed a novel enzymatic tissue digestion method,

suitable for the analysis of these antileishmanial drugs. The digestion method, baes on collagenase A incubation overnight (± 16 hours) at 37°C, led to completely dissolution of full thickness human skin biopsies and acceptable recovery of the drug analytes. Final extracts were injected on a Gemini C18 column for both miltefosine and amphotericin B, using alkaline eluent for separation and elution with miltefosine assays and acidic eluent for amphotericin B. Ion-pair chromatography was performed on an UPLC column for separation of paromomycin, using heptafluorobutyric acid as ion-pair reagent. Quantification was performed using a quadrupole – linear ion trap mass spectrometer. The methods were validated following FDA and EMA guidelines over linear calibration ranges of 4-1000, 10-2000, and 5-1000 ng/mL for miltefosine, amphotericin B and paromomycin, respectively. Validation parameters were all within internationally accepted criteria, including intra- and inter-assay accuracies and precisions within± 15% and ≤ 15% (within± 20% and ≤ 20% at the lower limit of quantitation). Patient human skin tissues were measured using the concentration described by the calibration curves in ng/mL. Conversion of skin tissue concentrations of the drugs was performed using the measured concentration times the added digestion solution in mL, and eventually divided by the mass of the skin tissue sample in mg to get the concentration µg/g skin. The lowest and highest concentrations measured for miltefosine, amphotericin B and paromomycin in patient skin tissue were respectively 1.7-117.9 µg/g, 11.8-2030 µg/g, and 3.4-51.5 µg/g, Using the developed methodologies human skin tissue samples were successfully quantified in skin biopsies from PKDL patients treated in India, Bangladesh and Sudan.

Keywords TREATMENT; ANTILEISHMANIAL; LC-MS/MS; LEISHMANIASIS

P4-016: PROTOCOL FOR A RANDOMISED, OPEN LABEL MULTICENTRE, NON-INFERIORITY CLINICAL TRIAL FOR NEW TREATMENT MODALITIES FOR CUTANEOUS LEISHMANIASIS CAUSED BY *Leishmania tropica*

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Cutaneous leishmaniasis (CL) is a neglected tropical skin disease, caused by the protozoan Leishmania. Although not a fatal disease, skin lesions often develop into ulcerating, disfiguring wounds and scars causing psychosocial suffering due to stigmatisation and discrimination. In Pakistan, CL is highly endemic and Leishmania tropica is the predominant species in Balochistan and Khyber Pakhtunkhwa provinces. Since decades, the mainstay treatment for CL is with pentavalent antimonial drugs, which are given in long courses (3-6 weeks) of painful injections. This antimonial treatment is contra-indicated for various vulnerable groups (pregnant women and patients with underlying morbidities), due to the potential toxic side effects. Besides that, this treatment is scarcely available in Pakistan public hospitals, and in addition has important financial and gender barriers to access treatment. Médecins sans Frontières has five CL diagnostic and treatment centres in the

two endemic provinces. Topical thermotherapy by radiofrequency generated heat, and oral miltefosine are effective in several Leishmania species, however these treatments have limited evidence for effectiveness in CL caused by L. tropica. Thermotherapy requires a single treatment session and miltefosine is an oral treatment and can be provided at primary health care level. The combination of these two treatments could shorten the treatment duration of miltefosine and have an additive effect from their different modes of action. The study is aimed to evaluate the effectiveness and safety of the thermotherapy (ThermoMed™), miltefosine (Impavido®) and the combination of the two treatments, in two cities with high prevalence of CL caused by L. tropica. We aim to find a treatment similar or better than the standard of care with intralesional injections of antimonial treatment (Glucantime®). We will perform a randomised, open label, multicentre, non-inferiority clinical trial (RCT), evaluating the efficacy and safety of new treatment options in four study arms: 1) topical thermotherapy (ThermoMed®, radiofrequency generated heat of 50°C, 30 seconds application, one session); 2) oral miltefosine capsules (2.5 mg/kg, 28 days); 3) a combination of thermotherapy (one session) and miltefosine (21 days); and 4) compared to I usuallthe standard of care with eight sessions (bi-weekly) of intralesional injections (local into the CL lesions) with meglumine antimoniate. We will recruit 832 CL patients (208 per study arm), aged ten years or older, and have with a parasitologically confirmed CL diagnosis, in two CL treatment centres in Quetta and Peshawar, Pakistan. Primary endpoints are initial cure rate (re-epithelisation and flattening of lesions) at day 91, and severity, seriousness and frequency of adverse events by treatment group. A descriptive analysis is followed by logistic regressions to analyse possible associations between the treatment and dichotomous primary outcomes of final cure/failure. We hope to identify an affordable, safe and effective treatment for CL caused by L. tropica. If successful, it can be implemented in primary healthcare facilities and increase treatment accessibility for CL patients.

Keywords *Leishmania tropica*; MILTEFOSINE; THERMOTHERAPY; PAKISTAN

P4-017: NEW DRUG COMBINATIONS FOR THE TREATMENT OF VISCERAL LEISHMANIASIS

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Current treatments for human leishmaniasis present several problems related to the development of resistance, side effects, high cost, poor oral bioavailability, chemical instability and prolonged treatments. Therefore, in the absence of an effective vaccine, there is a need for research into new drug treatments to overcome these problems. Drug repurposing and the combination of drugs with different mechanisms of action, which allow the reduction of the drug administered, thus reducing side effects and treatment times, are valid and cost-effective approaches to incorporate new treatments for these diseases. In a recent published screening of two commercial collections of 1,769 replacement drugs using splenic explants from mice infected with a strain of *L. donovani* with infrared fluorescence[1], 42 compounds with antileishmanial activity < 1µM were selected. From these compounds, we have chosen Nifuratel (NFT), a synthetic nitrofuran whose potency and selectivity point it as a promising oral drug for the treatment of visceral leishmaniasis. In addition, NFT, administered by the intralesional route, produced complete parasitological clearance against cutaneous model of *L. major* cutaneous leishmaniasis[2]. We have made several combinations of NFT with two drugs already used in the treatment of visceral leishmaniasis, miltefosine (MTF) and paromomycin (PMM), in order to reduce the doses of both drugs and, therefore, their potential toxic effects. To this end, we exposed either axenic amastigotes (isolated from the bone marrow of infected mice) or intramacrophagic amastigotes (obtained from primary cultures of murine spleen explants) both isolated from Balb/c mice infected with an infrared strain of *L. donovani*, to combinations of NFT/MTF and NFT/PMM in proportions 1/10 to 1/60 and 1/100 to 1/300,

respectively. Combination results were analysed with the Calcusyn statistic program[3]. After observing a clear antileishmanial synergy in both combinations, in vivo experiments in a model of chronic visceral leishmaniasis were performed with the combination of NFT + MTF both by oral administration, according to the following regimen 50 mg/kg/day NFT + 10 mg/kg/day MTF for 10 consecutive days. These experiments were performed with a strain of *L. donovani*-luc that allowed us to observe the development of the infection through the IVIS Spectrum image recording system. The administration of 50 mg/kg/day NFT produced a 55% parasitic burden reduction, while the reduction after the treatment with 10 mg/kg/day MTF was estimated to be > 85%. Interestingly, the effect after combination of both drugs was slightly superior to that found for MTF (10 mg/kg/day) alone (>90 %). Therefore, further combination regimens as well as combination of NFT with PMM, are needed to determine whether the combination of both drugs can have a clear antileishmanial effect *in vivo*.

Keywords COMBINATION THERAPY; EX-VIVO SPLENIC EXPLANT PLATFORM; IN VIVO IMAGING; NIFURATEL

P4-018: SPATIOTEMPORAL ANALYSIS OF ENDEMIC AND EPIDEMIC PATTERNS IN THE OF HUMAN VISCERAL LEISHMANIASIS IN A SMALL MUNICIPALITY IN SOUTHEASTERN BRAZIL

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Human visceral leishmaniasis is a severe systemic infectious disease, with a wide geographic distribution and a high morbidity and mortality rate. It constitutes a public health problem in tropical and subtropical regions. Visceral leishmaniasis has shown endemic patterns and episodes in urban areas, however, there are still gaps in knowledge with regards to disease transmission. Objective: This study aimed to analyze the spatiotemporal dispersion of visceral leishmaniasis cases in the municipality of Araçuaí, Minas Gerais. A study spatiotemporalof confirmed visceral leishmaniasis cases was conducted. This study focuses on the disease patterns of cases notified to the Notifiable Diseases Information System from the municipality of Araçuaí. The cases were separated into two periods: 2012 to 2014, characterized as an endemic period, and 2015 to 2017, epidemic period. The incidence rate was calculated, and for spatial analysis, the kernel map, directional distribution ellipse, and space-time scanning techniques were used. The correlations between visceral leishmaniasis cases and exposure variables (precipitation, humidity, and temperature) were calculated. Between 2012 and 2017, 68 new cases of visceral leishmaniasis were reported in residents of the Araçuaí. Of these, 20 cases occurred during the

endemic period (2012–2014) and 48 occurred during the epidemic period (2015-2017). The mean incidence of visceral leishmaniasis in the endemic period was 18.5 (95% confidence interval (CI) 5.9-32.5) and 44.4 in the epidemic period (95%CI, 12.0-28.6) by 100,000 inhabitants. The relative risk for the epidemic period was 2.4 (95% CI 1.4-4.1) when compared to the endemic period. A higher incidence of the disease was observed in rural areas. Kernel mapping analysis revealed hotspots in the urban area of the municipality. The directional distribution ellipse encompasses the urban perimeter and part of the rural area of the municipality, expanding eastward during the epidemic period. Spatial analysis revealed a high-risk cluster in rural areas. A positive correlation was observed between visceral leishmaniasis cases and temperature during the endemic period. Both the prevalence and incidence depend on understanding the different forms of the disease associated with geographically isolated transmission cycles and regional differences in surveillance. With regards to the expansion, some studies also indicate that the disease may be associated with the low impact of the control measures employed, possible improvement of the diagnosis and notification system, and people's mobility. Spatial analysis allowed us to outline the epidemiological scenario of human cases of visceral leishmaniasis in the municipality during the endemic and epidemic periods. The number of visceral leishmaniasis cases in Araçuaí remains high, considering its incidence in Brazil. It is distributed in the urban and rural areas of the municipality, with expansion during the epidemic period. These results suggest that ideal conditions for establishing and maintaining transmission are found in these locations. The pattern of occurrence of visceral leishmaniasis is not static, and the disease may expand to other areas of the municipality. These findings may be useful in case surveillance and in the work of health professionals and managers as well as in guiding further research.

Keywords VISCERAL LEISHMANIASIS; SPATIO-TEMPORAL ANALYSIS; ENDEMIC AND EPIDEMIC PERIODS

P4-019: ESSENTIAL OILS AND THEIR CONSTITUENTS AS SKIN PENETRATION ENHANCERS OF ANTILEISHMANIAL DRUGS

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Topical treatments could be useful in non-complicated cutaneous leishmaniasis (CL) cases. For most topically applied pharmaceuticals, penetration through the skin barrier is essential for developing their effects. For example, a transdermal treatment is necessary in cases of CL lesions where parasites live intracellularly on dermal macrophages. Essential oils (EO) and major metabolites derived from plants (MDP) could increase skin penetration of both lipophilic and hydrophilic drugs by interacting with the *stratum corneum*. This work aimed to determine the ability of EO and MDP derived from Colombian plants as permeation enhancers. Eight essential oils (EO1-5. EO8-9, EO19) and 12 major-metabolites derived from Colombian plants (MDP4,7,10,19,21, 22, 24, 31, 33, 35-37) were selected from the BioReto XXI-15:50 scientific program. Permeation studies using full-thickness mice skin were performed in Franz diffusion cells at 32 °C, following the OECD Test Guideline 428. The receptor chamber contained PBS buffer (pH 7.4). Caffeine hydrogels containing 1% w/v of EO or MDP were prepared. At various times over 24 h (1, 2, 4, 6, 24 h), 300 µL of the receptor was withdrawn and replaced with the same amount of fresh PBS buffer. Caffeine was analyzed by a UV-VIS spectrophotometry method at 272 nm. Once the experiments were completed histopathological analysis of the membrane was performed (n=8). We found an increase in the parameters of caffeine permeation in the presence of some EO and MDP. The value

produced by the steady-state flux (estimated by linear regression through the data obtained between one and 24 h) of caffeine gel (control) was 30±19.6 µgcm^{-2}h^{1}. An increase of almost 4-5 times was observed after being combined with EO2 or EO19. The values were 130±47.6 and 150±14.1 µgcm^{2}h^{-1} respectively. In addition, an increase of almost three times in caffeine flux was observed after MDP6, MDP19, and MDP22. The values were 86±21.0; 90±18.4 and 101±21.7 µg cm^{-2} h^{-1} respectively. The potency of caffeine's penetration alone (permeability coefficient Kp) was 800±503.5 cm^{-2} h^{-1}. An increase of almost 2-4 times was observed after being combined with EO2, EO19, MDP6, MDP19 where caffeine Kp values were 2900±1046.1, 1300±469.5, 2100±516.5, and 2200±973.9 cm^{2}h^{-1} respectively. Some changes in the skin epidermis were observed histopathologically at the end of the assay. The EO2, EO19, MDP6, and MDP19 tested were able to improve the permeation of caffeine through the skin layers, suggesting that these compounds may be effective for transdermal delivery of hydrophilic antileishmanial drugs.

Keywords CUTANEOUS LEISHMANIASIS; ESSENTIAL OILS; TRANSDERMAL DRUG DELIVERY; COLOMBIAN PLANTS

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P4-019.1: IN SILICO MOLECULAR DOCKING STUDIES AND ANTILEISHMANIAL ACTIVITY OF FRACTIONS *Malachra alceifolia* AGAINST *Leishmania mexicana* PROTEASES

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Malachra alceifolia Jacq (family Malvaceae), known "Malva" medicinal plant that is used as a traditional therapy in many regions of América, W. tropical África, and Tropical Asia. Traditionally this plant used in the form of extracts, powder, paste by populations from the northern Colombian for treating fever, stomach, inflammations and parasites. The extraction and chromatographic fractionation leaves extracted by maceration in 98% ethanol (15 L) for 4 days, extracts were chromatographed using open column fractionation on silica gel (16g; column length: 11cm; internal diameter: 2.4cm) using solvent mixtures of increasing polarity as follows: (Hexane/CHCl3, CHCl3, CHCl3/EtOAc, EtOAc, and EtOAc/MeOH). The identification of the components of fractions with major activity biological, an Agilent Gas Chromatograph 7890A series (Agilent Technologies, Inc., Santa Clara, CA, USA) was used. This study evaluated the in silico molecular docking performed by AutoDock Vina inhibitory ability of compounds bioactive fractions present over protein targets *Leishmania mexicana*; leishmanicidal activity axenic amastigotes *Leishmania mexicana pifanoi* (MHOM/VE/60/Ltrod) and cytotoxic activity in the RAW 264.7 murine macrophage cell line. The chemical analysis fractions of *M. alceifolia* leaves revealed bioactive fractions, MAF8C and MAF9-10 with secondary metabolite presence unreported in the literature, alpha-Tocospiro A, alpha-Tocospiro B, gamma-Tocopherol, Alpha-Amyrin, and Methyl commate A in this genus. The docking study provided an insight into the prediction of affinity, activity, binding, and orientation of alpha-amyrin to the target

protein Pyruvate kinase of *L. mexicana* 9.9 Kcal/mol. All fractions showed high for antileishmanial activity against *L. mexicana*, MAF8C and MAF9-10 at 50 µg/mL percentages of survival 4.60% and 16.2%. The MTT assay revealed that bioactive fractions of *M. alceifolia* exerted no significant cytotoxicity in the RAW264.7. The *M. alceifolia* has the potential of active phytoconstituents, which can be used to search for new drugs and molecular targets, Alpha-Amyrin was the compound that showed the best free binding energy shown to have good antileishmanial. Furthermore, further contributions to research, validation, and conservation of traditional knowledge of medicinal plants globally are needed. This research was supported the University of Cartagena, Doctoral program in biomedical sciences from the University of Cartagena and the National Program for Doctoral Formation Minciencias, 727- 2015, Colombia.

Keywords: *Malachra alceifolia;* PHYTOCONSTITUENTS; ANTILEISHMANIAL ACTIVITY

5.3. DRUG DISCOVERY & DEVELOPMENT

P2-025: IN VITRO TRIVALENT ANTIMONIAL SUSCEPTIBILITY AMONG *Leishmania (viannia) panamensis* CIRCULATING IN PANAMA: PILOT STUDY

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In Panama, leishmaniasis is one of the most prevalent parasitic diseases and one of the main public health problems, mainly affecting populations in rural areas. *Leishmania (Viannia) panamensis* is the most frequently etiologic agent of cutaneous leishmaniasis. The treatment involves the use of pentavalent antimonial that is the first-line drug used in the treatment of leishmaniasis in Panama. Although these drugs have successful cure rates, some reports suggest that some strains of *Leishmania* sp. are resistant to these drugs. In Panama a biodiversity of strains causes infection in human, however rare studies analyze the susceptibility of *Leishmania (Viannia) panamensis* to the antimonials. Therefore, it is important to standardize and apply methodologies that allow evaluating the antimonial susceptibility among *Leishmania (Viannia)* parasites circulating in Panama. The susceptibility to trivalent antimonial (SbIII) was evaluated in 11 isolates of

L. (V.) panamensis obtained from patients with cutaneous leishmaniasis from different endemic areas of the country between 2015 to 2021. Promastigote forms were incubated with 200-6.25 µg/mL of trivalent antimonial, and the parasite viability was determined at 24, 48 and 72 hours. To analyze amastigote susceptibility to trivalent antimonials, J774A.1 murine macrophage cell-line was infected with promastigote forms of the 11 isolates, and infected cells were treated with trivalent antimonial at 0.01–0.0001 µg/ml. The susceptibility of amastigotes to trivalent antimonials was analyzed at 24, 48 and 72 hours of infection by determining the effective concentration 50 (EC_{50}). The data was analyzed using the GraphPad Prism software, version 5.0. The range of sensitivity of the promastigote form varied from 6.594 - 9.151 µg/ml, and for the amastigote form varied from 0.002351 - 0.0002284 µg/ml, suggesting that the promastigote form is less susceptible to SbIII over time (p=0.009). Compared to the *L. (V.) panamensis* reference strain, it was observed that two clinical isolates were less susceptible to the drug (p=0.02). One of these isolates, did not show an increase in its susceptibility to SbIII as time increased, unlike the other isolates. Before drawing conclusions about antimonial susceptibility among *Leishmania (Viannia)* parasites in Panama, other evaluations are necessary.

Keywords *Leishmania Viannia panamensis*; SBIII; DRUG SUSCEPTIBILITY; J774A.1; PANAMA

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P2-027: ACTIVITY OF 1,2,4-TRIOXOLANE AND 1,2,4,5-TETRAOXANE ENDOPEROXIDES AGAINST OLD-WORLD *Leishmania* SPECIES

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Visceral leishmaniasis caused by *Leishamania donovani* and *L. infantum* can be fatal in untreated. Current treatment for visceral leishmaniasis is difficult due to a lack of effective, non-toxic, and non-extensive medications. Artemisinin and derivatives as well as synthetic peroxides have demonstrated efficacy against *Leishmania* spp. A significant advantage of synthetic endoperoxide-containing compounds such as 1,2,4-trioxolanes and 1,2,4,5-tetraoxanes is their accessibility, which enables the preparation of chemically diversified libraries of analogues and a more precise selection of a possible lead compound. This study aimed to evaluate the selectivity of 12 synthetic endoperoxides (1,2,4-trioxolanes; 1,2,4,5-tetraoxanes) and uncover their biochemical and morphological effects on *Leishmania* parasites and to provide an insight into the possible mode of action by analysing ultrastructural morphological alterations, oxidative stress and events that lead to parasite death. The compounds were screened for in vitro activity against *L. infantum* and *L. donovani* and for cytotoxicity in two monocytic cell lines (J774A.1 and THP1), using MTT assay. Ultrastructural changes induced by the compounds were screened by TEM and reactive oxygen species formation, phosphatidylserine (PS) externalization, and

mitochondrial impairment were measured by flow cytometry. Results have shown that peroxides exhibited fair to moderate micromolar anti-*Leishmania* activity against promastigotes and amastigotes of the two *Leishmania* species. Tetraoxane LC132 and LC138 demonstrated good leishmanicidal activity on *L. infantum* amastigotes (13.15 and 23.94 μM) and the later with low cytotoxicity towards mammalian cells (SIs 22.1 and 118.6). LC138 was able to induce late apoptosis and dose-dependent oxidative stress. Ultrastructural analysis showed vacuolization, flagellar pocket, and membrane disarrangement, which compromise viability. One of the key features of these molecules relates to the role of the peroxidic bridge, which is believed to play a role in the mechanism of action, as indicated from prior studies on the effect of artemisinin and its semisynthetic derivatives in *L. infantum*. LC138 presented mild evidence of PS externalization, which was more evident in a late stage of apoptosis (48h), though with a generation of reactive oxygen species (ROS) at long exposure times (24h). These evidences indicates that LC138 causes oxidative stress, which is consistent with late mitochondrial injury. The results obtained for *Leishmania* activity and safety of LC132 and LC138, together with their easy access through chemical synthesis, support the relevance for further investigations, namely in vivo testing, of this class of compounds in the context of visceral leishmaniasis therapy.

Keywords *Leishmania*; ENDOPEROXIDES; SAFETY PROFILE; REACTIVE OXYGEN SPECIES; ULTRASTRUCTURAL CHANGES

Financing:} GHTM (UID/Multi/04413/2019; IF/00743/2015/CP1320) CCMAR (UID/Multi/04326/2013); SFRH/BD/130407/2017

P2-028: ACTIVITY OF BIOACTIVE ISOFLAVANS FROM *Tabebuia chrysantha* TIMBER BY-PRODUCTS AGAINST *Leishmania braziliensis* AND *Trypanosoma cruzi*.

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Timber by-products are an exciting and emerging source of secondary metabolites. In the search for new antiparasite molecules and through bio-guided in vitro assays, high activities in the ethanolic extract (SQB-11) from *Tabebuia chrysantha* sawdust were detected against *Leishmania braziliensis* and *Trypanosoma cruzi*. Through a chromatographic separation, five fractions and two pure isoflavans (sativan and vestitol) were obtained and analyzed against these parasites. Fraction S4 showed high activity against *L. braziliensis* and *T. cruzi* at EC_{50} of 11.1 µg/mL and 5.1 µg/mL, respectively. In turn, sativan and vestitol metabolites was highly active against both parasites with EC_{50} values lower than 10 µg/mL (34.92 µM and 36.7 ⍰M, respectively. Nevertheless, fractions and metabolites showed high cytotoxicity with LC_{50} values between 7.3 µg/mL and 60.8 µg/mL, except for fraction S8 which showed moderate cytotoxicity with LC50 of µg/mL. The effectiveness of the mixture of sativan and vestitol was confirmed in hamsters with experimental cutaneous leishmaniasis caused by *L. braziliensis* and mice infected with *T. cruzi*. So, the infected hamsters treated with the 3% mixture of sativan and vestitol (30 days) showed 67% of clinical cure, and the remaining 37% of hamsters showed a reduction of their lesion size between 30 and 70%. In addition, mice infected by *T. cruzi* and treated with a mixture of sativan and vestitol (100 mg/kg/day, 25 days) showed a 75% reduction of parasitemia. These results demonstrate the activity of *T. chrysantha* against *L. braziliensis* and *T. cruzi* infections and highlight the

pharmacological potential of waste from the wood industry, which has tons of valuable chemicals for the development of new antiparasite drugs.

Keywords CUTANEOUS LEISHMANIASIS; CHAGAS DISEASE; ANTIPARASITE ACTIVITY; CYTOTOXICITY

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P2-030: TRYPANOCIDAL ACTIVITY AND CYTOTOXICITY OF ENT-BEYERENE DERIVATIVES

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There are no vaccines available to prevent infection by Trypanosoma cruzi, and the therapeutic options are reduced to two drugs: benznidazole and nifurtimox. Therefore, it is urgent to discover molecules with activity against the parasite and from which an effective drug can be developed. In previous work, the ent-Beyer-15-en-19-ol (beyerenol) and some derivatives showed high in vitro activity against intracellular amastigotes Leishmania braziliensis parasites. Nonetheless, their biological activity against T. cruzi has not been determined, nor has the relationship between their structure and activity. Therefore, in this study, 18 compounds derived from ent-Beyerene were synthesized, and their trypanocidal activity and cytotoxicity were evaluated. Compounds (2), (4), (7), (11), (12), (13) and (19) were active with an EC_{50} < 25 µM, of which (11), (12), and (19) were the most active with EC_{50} values of 6.1 µM, 5.4 µM, and 4.8 µM respectively. The Index of selectivity of these most active compounds ranged between 2.5 and 3.5 and compound (19) was 14 times more active than benznidazole. These compounds exhibit unsaturation at C15-C16 and relatively bulky groups at C-19. These ent-beyerenes derivative active compounds were obtained from steviol, a natural, abundant, renewable, and low-cost source. Therefore, generating a large amount of raw material increases the possibility of obtaining active molecules against a disease with a high morbidity and mortality burden. Likewise, their synthesis did not require reagents or methodologies different from those commonly used in organic synthesis, which will result in more significant advantages to obtaining more affordable drugs.

Keywords *Trypanosoma cruzi*; CHAGAS DISEASE; ENT-BEYERENES DERIVATIVES; TOXICITY; STEVIOSIDE

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P2-031: MENTHOL, EUGENOL AND THYMOL CARBONATE DERIVATIVES AS POTENT ANTIPARASITIC AGENTS: SYNTHESIS AND *IN VITRO* STUDIES ALONG WITH COMPUTER-AIDED APPROACHES

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Despite the number of deaths and the significant economic and social costs associated with Chagas diseases and, leishmaniasis worldwide, available drugs are limited and have serious side effects and high toxicity for patients. Therefore, there is an urgent need for safe, low-cost, and effective treatments. Natural products are an important source of bioactive

compounds and there is current interest in finding natural bioactive molecules that can be used for treating these parasitic diseases. In the present study we proposed to evaluate the *in vitro* antiparasitic activity of new menthol, eugenol and thymol derivatives against *Trypanosoma cruzi* and *Leishmania braziliensis. In silico* physicochemical and pharmacokinetic properties of synthesized compounds were also determined; moreover, we propose to explore their mode of action through molecular docking against TcDHODH, LbDHODH. Several series of carbonate derived compounds were synthesized from menthol, thymol and eugenol using different aliphatic alcohols and N, N-carbonyldiimidazole. Spectroscopic techniques, including 1H nuclear magnetic resonance (NMR), 13C NMR, FTIR and high-resolution mass spectrometry (HRMS) were used to confirm the structures of the synthesized compounds. The cytotoxicity of the compounds was assessed using U-937 cells. *In vitro* trypanocidal, and leishmanicidal antiplasmodial activities were evaluated using a *T. cruzi* and *L. braziliensis* organisms, respectively. In addition, *in silico* studies were also performed through molecular dynamics simulations and MM-PBSA analysis. The assay revealed that most of the menthol derivative compounds were highly active against intracellular amastigotes of *T. cruzi* and *L. braziliensis* while eugenol derived compounds showed high activity against *L. braziliensis* and moderate activity for *T. cruzi*. Contrary, both, thymol and carbonate derivatives although were cytotoxic to mammal U-937 cells, they were highly active against *T. cruzi* but the activity against *L. braziliensis* was low. The prediction of the ADME properties suggests that all the compounds have drug-like molecular properties and the probability to be lead candidates. Finally, molecular dynamics simulations, and MM-PBSA studies indicate that menthol at the substrate binding site of TcDHODH and LbDHODH is structurally stable in the same order as the natural substrate; also, interactions of menthol with residues involved in the inhibition of TcDHODH protein was predicted. The docking results also showed that eugenol has binding energy similar to the natural substrate. The present study demonstrates that menthol, eugenol and thymol carbonate derivatives have promising therapeutic potential as trypanocidal and leishmanicidal agents; nonetheless further studies are needed to validate their efficacy as antiparasitic drugs in *in vivo* assays and the mechanisms of action proposed in this study need to be experimentally verified by future enzymatic assays.

Keywords PRODRUGS; CYTOTOXICITY; MOLECULAR DYNAMICS; LEISHMANICIDAL ACTIVITY; TRYPANOCIDAL ACTIVITY; NATURAL PRODUCTS

Financing Universidad Nacional de Villa Maria, Córdoba-Argentina and Universidad de Antioquia, Medellín-Colombia

P2-032: IMMUNOMODULATORY EFFECT OF P2Et FRACTION OF *CAESALPINIA SPINOSA* (MOLINA) KUNTZE ON THE RESOLUTION OF *Leishmania (v.) braziliensis* INFECTION I*N VITRO.*

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The treatment of cutaneous leishmaniasis continues to present many disadvantages worldwide: such as loss of efficacy, poor adherence to treatment, due to the use of high doses and long-term regimens, and high toxicity, in addition to parasite drug resistance or decreased sensitivity. All this makes evident the need to search for new treatments and, mainly, hear the call of the WHO to use traditional medicine and phytochemical research. In this work was studied the immune response modulation of an ethanolic extract of the native Colombian plant *Caesalpinia spinosa* (Molina) kuntze, which has been shown in previous studies to have a leishmanicidal activity, as well as induction of proliferation, migration of cells to improve wound healing. Despite this, its mechanism of action and its possible specific immunomodulatory effect are still unknown. Assays were performed on differentiated macrophages from monocytes of the U937 line infected with the wild type *L. braziliensis* strain (MHOM/CO/88/UA301-wt). Soluble mediators TGF-β1, CCL22 and IL-4 cytokines were assessed to demonstrate the predominance of a type of profile in the immune response, regarding nitric oxide levels as the main intermediate at the macrophage level, at 12, 24 and 48 hours of production, using the fraction as an experimental treatment condition at an effective concentration (EC_{50}) of 11.51 µg/mL, proposing an approximation to the mechanism of action of this plant extract.

Keywords *Leishmania braziliensis*; CUTANEOUS LEISHMANIASIS; IMMUNE RESPONSE; IMMUNOMODULATION; TREATMENT

P2-033: IDENTIFICATION OF *Leishmania* PROTEASOME 20S INHIBITORS THROUGH TARGET-BASED VIRTUAL SCREENING AND PHENOTYPIC SCREENING

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Proteasomes are protein complexes that act by degrading unneeded or damaged proteins into small peptides and recycled as amino acids. Inhibition of proteasomes leads to the accumulation of ubiquitinated proteins and vesicles, triggering autophagic death pathways. Recent advances reinforce the importance of proteasome 20S as a selective molecular drug target in trypanosomatids, including *Leishmania* parasites. In partnership with Atomwise and as part of the Artificial Intelligence Molecular Screening – AIMS Program, here we pre-selected putative antileishmanial compounds driven by Atomwise's proprietary AtomNet® technology that uses a deep learning neural network for drug discovery through protein - small molecule binding predictions. For target-based hit discovery, a library of several million compounds was virtually screened against *Leishmania* proteasome 20S (PDB ID: 6QM7), considering the β3-5 chains that encompass the ligand binding site. A diverse set of 83 high-scoring predicted hits were obtained, and their antileishmanial activity was initially tested *in vitro* against *Leishmania infantum* intracellular amastigotes. The 20 most promising compounds were then tested against *L. braziliensis* and *L. amazonensis* intracellular amastigotes. Eight compounds were highly potent and selective against the parasites, presenting IC_{50} values varying from 0.3 to 4.8 µM and selective indexes higher than 288-fold. Most of the selected compounds presented CC_{50} values over 100 µM on THP-1 derived human macrophages. Cell-based proteasome 20S activity assay will be performed to validate proteasome inhibition by measuring

chymotrypsin-like, trypsin-like and caspase-like protease activities associated with proteasome in axenic conditions. Since structural analysis revealed similarities among proteasome 20S from different trypanosomatids, these hit compounds could also be tested against human and animal trypanosomiasis. Joining efforts between private sector and public partners can accelerate the collaborative drug discovery process towards the identification of novel agents to tackle neglected diseases. In this way, Atomwise, Fiocruz and strategic partners are devoted to find new antileishmanial agents to improve the drug arsenal against leishmaniasis.

Keywords *Leishmania*; PROTEASSOME 20S INHIBITION; AIMS PROGRAM; DRUG DISCOVERY; ATOMWISE

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P2-035: 6-BROMO-2´-DE-N-METHYLAPLYSINOPSIN: AN ANTI-*Trypanosoma cruzi* HIT COMPOUND ISOLATED FROM THE CORAL *Tubastraea tagusensis*

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Chagas disease is a challenging disease, affecting seven million people worldwide and lacks effective drugs. In this context, marine natural products represent a huge chemo-diverse space for the selection of new hit compounds against protozoan parasites. In this work, we isolated the indole alkaloid 6-bromo-2´-de-N-methylaplysinopsin (BMA) from the coral *Tubastraea tagusensis* and evaluated the activity against trypomastigotes and intracellular amastigotes of *Trypanosoma cruzi* (Y strain). The IC_{50} values resulted in 62 µM and 5 µM, respectively, with no mammalian cytotoxicity to fibroblasts up to 200 µM. Using MALDI-TOF/MS and fluorescent-based techniques as flow cytometry, we studied the lethal action of BMA. The compound induced no alterations of the plasma membrane permeability but caused a rapid depolarization of the mitochondrial membrane potential, affecting the ATP levels. Using acridine orange and Fluo 4AM, we observed a pH alteration of the acidocalcisomes, associated to an imbalance of the calcium levels. The mass spectral differences between the parasites treated with BMA and the clinical used drug benznidazole, suggested a differential mechanism for the alkaloid. The SwissADME *in silico* platform, suggested acceptable drug-like properties, indicating this compound as a promising prototype for future chemical optimizations.

Keywords *Tubastraea tagusensis;* MARINE INVERTEBRATE; *Trypanosoma cruzi;* MECHANISM OF ACTION; 6-BROMO-2´-DE-N-METHYLAPLYSINOPSIN

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P2-036: EFFECTS OF SERINE PROTEASE INHIBITORS ON *Leishmania infantum*

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Leishmaniasis chemotherapy exhibit limitations, thus is very important to search new compounds with leishmanicidal activity. Serine proteases are involved in the pathogenesis of leishmaniasis. Based on that, this work had as objective evaluate the *in vitro* and *in vivo* effects of PF-429242 and TPCK, two well-known serine protease inhibitor, against *Leishmania infantum*. The effect of the compounds in promastigote forms and in macrophages was determined by MTT colorimetric method. The amastigotes viability was analyzed by counting intracellular parasites after Giemsa staining. PF-429242 and TPCK-induced ultracellular changes in promastigotes and intracellular amastigotes of *L. infantum* were determined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The *in vivo* effect of PF-429242 and TPCK was determined in BALB/c mice infected with *L. infantum* and treated for 10 days with 25 mg/kg intraperitoneally. *In vivo* toxicity was evaluated by determination of creatinine, AST (aspartate aminotransferase), and ALT (alanine

aminotransferase) levels in the serum of *L. infantum*-infected BALB/c and treated with TPCK, using laboratory kits colorimetric kinetic test. PF-429242 and TPCK exhibited low toxicity against mammalian cells (CC_{50} = 189.07 and 138.80 µM, respectively) and were active against *L. infantum* promastigotes (IC_{50} = 2.78 and 11.34 µM, respectively) and intracellular amastigotes (IC_{50} values = 14.07 and 21.72 µM, respectively), displaying selectivity for parasite when compared to host cells. The ultrastructural evaluation by TEM showed that promastigotes treated with PF-429242 presented alterations in mitochondria and flagella, vacuoles, autophagic bodies and increase of lipid droplets. *L. infantum* intracellular amastigotes treated with PF-429242 presented mitochondrial alteration, myelinic figures and endoplasmic reticulum profiles surrounding the parasitophorous vacuole. Analyzes by TEM revealed an increased number of cytoplasmic vacuoles and some vacuoles containing electron-dense and membranous materials in *L. infantum* promastigotes and intracellular amastigotes treated with TPCK. In addition, after treatment with TPCK, SEM analyzes showed *L. infantum* promastigotes with flagellar changes. The treatment with PF-429242 (25 mg/Kg) of BALB/c mice infected with *L. infantum* not altered the parasite load in liver and spleen, however, the *in vivo* treatment with TPCK (25 mg/Kg) reduced significantly the parasite load in liver and spleen. TPCK did not altered creatinine, AST and ALT levels in the serum of infected animals, thus this compound not induce renal and hepatic acute toxicity in treated animals when compared to animals untreated. The *in vitro* and *in vivo* effect of PF-429242 and TPCK on *L. infantum* as well as its mechanism of action were demonstrated for the first time during this work. Flagellar alterations induced by PF-429242 and TPCK may be related to the inhibition of subtilisin, a serine protease, as it has already been described that subtilisin-deficient *L. donovani* amastigotes display a retained flagellum. This work highlights effects of PF-429242 and TPCK against *L. infantum* and confirms the importance of serine proteases as drug targets in *Leishmania* spp.

Keywords SERINE PROTEASES; PF-429242; TPCK; LEISHMANIASIS; *Leishmania infantum*

Financing FAPERJ, CAPES and CNPq

P2-037: ANTILEISHMANIAL SAR STUDY OF NEW 3-NITROIMIDAZO[1,2-*A*]PYRIDINES BIOACTIVATED BY NTR1: IDENTIFICATION OF NEW POTENT COMPOUNDS WITH IMPROVED PHYSICOCHEMICAL AND PHARMACOKINETIC PROPERTIES

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Leishmaniases are vector-borne parasitic diseases caused by several species of flagellated protozoa of the genus *Leishmania*. More than 1 billion people in 98 countries are at risk of infection, and nearly 1 million new cases occur annually. Of the three main forms of the disease, life-threatening visceral leishmaniasis (VL), with an estimated 50,000 – 90,000 new cases per year, cause more than 30,000 deaths annually. Unfortunately, in the absence of a vaccine, the treatment of VL is exclusively based on a small number of drugs that have significant toxicity, low efficacy, increasing drug resistance, high cost, non-oral route of administration and requiring prolonged hospitalization. Moreover, only a few new chemical entities have reached phase I clinical stage of development and only one molecule remains in phase II. Thus, new efficient, safe and inexpensive oral antileishmanial drugs are urgently needed. In this context, our team identified a first antileishmanial hit compound in 8-halogeno-3-nitroimidazo[1,2-*a*]pyridine series bearing a phenylsulfonylmethyl group at position 2 (Hit A). The introduction of a 4-chlorophenylthioether moiety at position 8 (Hit B) improved the *in vitro* antileishmanial activity (EC_{50} *L. infantum* ama. intra. = 3.2 µM) but showed limited aqueous solubility (thermodynamic solubility = 1.4 µM) and poor mouse liver microsomal stability ($t_{1/2}$ = 3 min). The sulfoxide and sulfone metabolites of Hit B were identified and showed to remain active, but were also rapidly metabolized *in vitro* ($t_{1/2}$ = 3 min). The

para position of the phenyl ring at position 2 was also identified as potentially oxidized. To improve antileishmanial activity, aqueous solubility and microsomal stability, we explored the structure-activity relationship of the substituents at theposition 2 of the imidazo[1,2-*a*]pyridine ring, including the probable oxidized metabolite, the analogues with metabolic blockers at the *para* position or by replacing the sulfone group by a sulfoximine group. Thus, 24 new compounds were synthesized using type 2 nucleophilic substitution (S_N2), nucleophilic aromatic substitution (S_NAr) and Suzuki-Miyaura cross-coupling reaction. Their influence on cell viability (cytotoxic concentration 50% = CC_{50}) was assessed on HepG2 and THP1 cell lines. The *in vitro* antileishmanial activity (measured by the effective concentration 50% = EC_{50}) was also measured on both the promastigote and axenic amastigote form of *L. donovani* and *L. infantum*, respectively. The active compounds were also tested on the intracellular amastigote form of *L. infantum*. Finally, the *in vitro* physicochemical and pharmacokinetic data of selected key compounds were evaluated. Some of the newly 2- substituted compounds showed improved antileishmanial activity, compared to the previous hit molecules, in particular the sulfoximine and *gem*-trifluoropropyl derivatives bearing a 4-chlorophenylthioether moiety at position 8 (EC_{50} *L. infantum* ama. intra. = 0.8 µM and 1.2 µM, respectively). However, only the *gem*-trifluoropropyl derivative showed better aqueous solubility (thermodynamic solubility = 12.4 µM) and improved microsomal stability ($t_{1/2}$ = 9 min) than Hit B. Finally, in order to improve aqueous solubility and microsomal stability, we replaced the 4-chlorophenylthioether moiety with a pyridin-4-yl substituent in position 8 of the *gem*-trifluoropropyl derivative. The antileishmanial activity, aqueous solubility and microsomal stability of the latter compound are in progress.

Keywords IMIDAZO[1,2-*A*]PYRIDINE; NITROAROMATIC; NITROREDUCTASES; *Leishmania donovani*; *Leishmania infantum*

P2-039: TEMPERATE ZONE PLANT NATURAL PRODUCTS – A NOVEL RESOURCE FOR ACTIVITY AGAINST TROPICAL PARASITIC DISEASES

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The natural world is host to a wide variety of biologically active compounds that can effectively treat a range of diseases. However, the search for plant-derived natural products with anti-parasitic activity have largely been focused on plants that are found where the parasites are endemic. As such, plants grown in temperate regions remain largely untested for novel anti-parasitic activities. Here, a natural product library containing over 600 purified compounds from temperate zone plants were screened for activity against *Leishmania mexicana* axenic amastigotes, *Trypanosoma evansi*, *Trypanosoma brucei* bloodstream form and *Plasmodium falciparum*. Initial screens revealed 65, 15, 18 and 6 compounds which decreased parasite viability by 50% or more at 1-2 μM. These initial hits were validated in concentration-response assays against the parasites and the human HepG2 cell line, in order to identify hits with EC50 <1 μM and a selectivity index of >10. Additional selectivity assessment was carried out against the activated THP1 cell line for *L. mexicana* hits. While no compounds were identified to fulfil these criteria for all four parasite species, a number of compounds were found to be effective against individual species. Four lanosterone-like

sterols with low cytotoxicity (CC_{50} of 28-57 µM in HepG2 and 17-27 µm in THP1) and nanomolar activity (EC_{50} of 0.2-0.5 µM) against *L. mexicana* axenic amastigotes were found to have similar activity against *L. donovani* axenic amastigotes (EC_{50} of 0.1-0.3 µM). An *L. mexicana* resistant line was generated for the sterol 700022 and was found to have cross-resistance with the other 3 sterols, suggesting a similar mode-of-action or entry mechanism. However, this resistance line was also found to have cross-resistance to the anti-leishmanial drug miltefosine. This study highlights the potential of a temperate plant secondary metabolites as a novel source of natural products against tropical parasitic diseases, as well as the importance of assessing cross-resistance of screen hits with current therapies.

Keywords DRUG DISCOVERY; NATURAL PRODUCTS; TEMPERATE ZONE; DRUG RESISTANCE

P2-040: IDENTIFICATION OF INHIBITORS OF CRUZAIN FROM *Trypanosoma cruzi* WITH POTENTIAL TRYPANOCIDAL ACTIVITY

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Chagas Disease (CD), caused by the protozoan *Trypanosoma cruzi*, is considered a Neglected Tropical Disease by the World Health Organization and affects approximately seven million individuals worldwide, with the highest number of cases in Latin America. Clinically, CD has two phases, of which the chronic phase is characterized by reduced efficacy in drug therapies. This and other factors make the development of new strategies that aim to identify molecules capable of becoming alternatives to or complement current chemotherapy vitally important. In this sense, considering the important role of cruzain from *T. cruzi* in key processes in the life cycle of this parasite, such as proliferation, differentiation and cell invasion, this protease has become an interesting molecular target to be explored. In view of the aforementioned, the present study aimed to identify cruzain inhibitors with potential trypanocidal activity. Initially, recombinant cruzain was obtained by means of heterologous expression in a prokaryotic system in the form of zymogen and sequentially activated by autoproteolysis, resulting in a protein with a molecular mass of approximately 23 kDa. Subsequently, in the search for potential inhibitors

of cruzain, a cystatin from the venom of *Austrelaps superbus* (AsCystatin) with inhibitory action on the catalytic activity of cysteine proteases was identified from previously published scientific literature. In order to evaluate the *in silico* interactions of this potential inhibitor with cruzain, a three-dimensional model was obtained by homology modeling, along with an interaction analysis through molecular docking. It was observed that key regions for the catalytic activity of cruzain interact with AsCystatin. Considering these results, it was decided to recombinantly express the potential inhibitor, obtaining a protein with a high degree of purity and a relative molecular mass of 13 kDa. Next, the inhibition of AsCystatin on the activity of cruzain (IAE_{50}) was evaluated, observing that approximately 20 µM of cystatin was able to inhibit 50% of the catalytic activity of the recombinant enzyme. Based on the *in silico* analysis performed previously, original and modified peptides were designed and tested, which allowed for the identification of four peptides with inhibitory capacity on the enzymatic activity of cruzain. Finally, three of these peptides showed trypanocidal activity on epimastigote forms of *T. cruzi* in *in vitro* models. In conclusion, in this study it was possible to identify AsCystatin and four peptides derived from this protein with inhibitory activity on cruzain, highlighting the cytotoxic effect associated with these peptides observed in *in vitro* assays on *T. cruzi*.

Keywords CHAGAS DISEASE; MOLECULAR TARGET; INHIBITORY ACTIVITY; PEPTIDES; ANTIPARASITIC ACTIVITY

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Financing Programa Nacional de Incentivo a los Investigador-PRONII-CONACyT; FOCEM; FAPERO, CAPES, CNPq

P2-041: TRYPANOTHIONE REDUCTASE AS A MOLECULAR TARGET FOR NEW INHIBITORS PROSPECTION: LAAO OF *Crotalus atrox* EVALUATION WITH ANTIPARASITIC ACTIVITY

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According to data from the World Health Organization (WHO), about 1 million new cases of leishmaniasis appear annually. Chemotherapy is based on ability of drugs to interfere with the parasite survival, with several limitations potentiating this neglected disease as a serious health problem. Approaches in the search for alternatives to treatment using molecular targets of *Leishmania* spp. are being developed by several research groups. Among these molecular targets are the enzymes involved in essential parasite pathways, such as trypanothione reductase (TR), involved in combating the oxidative stress generated by reactive species of oxygen produced by host macrophages during infection. This research aimed to investigate *Leishmania braziliensis* TR as a molecular target for prospection of new inhibitors and subsequent evaluation of the LAAO of *Crotalus atrox* with antiparasitic activity. The recombinant protein was expressed in its active, homodimeric form, with each monomer with approximately 54 kDa molecular mass and isoelectric point of 6.0. The analysis of the primary structure of the expressed enzyme, revealed a high percentage of identity with the TR´s of different trypanosomatids. The biodiversity prospection carried out with natural compounds derived from animal poisons, in order

to identify the potential inhibitors, the inhibitory capacity of the L-amino acid oxidase (LAAO) fraction of *Crotalus atrox* venom was evaluated against the recombinant molecular target (IC50:119 µg/mL). Subsequently, the *in vitro* leishmanicidal activity against promastigotes forms of *Leishmania amazonensis* (IC50: 1.8 µg/mL) and *Leishmania braziliensis* (IC50: 1.3 µg/mL) and sequentially, CC50 was determined on THP1 cells (291.38 µg/ml); J774.A1 (24.16 µg/ml); HepG2 (4.05 µg/mL) and VERO (1.95 µg/mL). Molecular modeling by homology of LAAO and TRLb, molecular docking between the two proteins and the virtual screening between the peptides derived from the structure primary linkage of LAAO with the parasite enzyme, was carried out. The *in-silico* studies allowed the identification and synthesis of 5 peptides which interacted in key regions of the enzyme. Additionally, 2 peptides with microbicidal activity were tested for evaluation of the inhibitory potential of TR. In this study, the leishmanicidal potential of the LAAO fraction from *C. atrox* venom and the peptide identification of ^{353}RFIYY^{357} sequence, which has *in vitro* inhibitory activity against TR (IC50: 287.5 µM), was demonstrate. The promising results obtained in this work suggest future research studies aimed at evaluating the leishmanicidal potential of the peptide and proposing structures derived from this inhibitor with the ability to add the effect antiparasitic.

Keywords LEISHMANIASIS; TRYPANOTHIONE REDUCTASE; SNAKE VENOMS; L-AMINO ACID OXIDASE

Financing PRONII-CONACyT; FOCEM; FAPERO, CAPES, CNPq.

P2-042: EFFECTIVENESS OF SYNTHETIC QUINOLINE ANALOGUES AGAINST CUTANEOUS LEISHMANIASIS (CL) INFECTION

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Cutaneous Leishmaniasis (CL) is considered a neglected disease with an estimated global incidence of 600,000 to 1,000,000 new cases each year, mainly affecting populations in more than 87 countries around the world, predominantly in Afghanistan, Pakistan, the Republic of Islamic Iran, Saudi Arabia, the Syrian Arab Republic, Algeria, Ethiopia, Brazil, Colombia and Peru. The increasing decrease in the efficacy of the drugs available for its treatment, added to the adverse effects and high costs of treatment, limit their use and make the disease a serious public health problem in the world. In this study, the synthesis and evaluation of the in vitro and in vivo antileishmanial activity of six quinoline analogs is reported, the synthesis was carried out using quinaldine and hydroxyquinaldine with different aromatic aldehydes, using acetic anhydride and heat as reaction medium, the structure of these compounds was corroborated by spectroscopic methods (NMR in one and two dimensions and IR), the *in vitro* antileishmanial activity was evaluated on the *Leishmania (V) panamensis* strain, using the flow cytometry method; cytotoxicity was evaluated on U-937 cells using the MTT method, taking into account the promising activity of the analogs (**1-8**), the therapeutic response *in vivo* was evaluated using golden hamsters (*Mesocricetus auratus*) experimentally infected with *L. (V) panamensis* and treated with a 1% cream formulation of the compounds. All compounds showed promising activity in vitro with mean effective concentration EC_{50} values ranging from 1.1µg/mL (3.8µM) to 7.10µg/mL (19.3µM). Likewise, treatment of hamsters with compounds **1-8**, treated topically for 20 days, produced improvement in most hamsters and even cure in some hamsters treated with compounds 2-4, 6 and 8. As expected,

treatment with meglumine antimoniate produced a cure in almost all the hamsters in this group. None of the treatments affected the weight of the hamsters nor did they affect the serum levels of AL, BUN and creatinine. These results confer therapeutic potential to this type of compounds, especially to compounds 2-4, 6 and 8 for the treatment of CL caused by *L. panamensis*. The effectiveness found in this work can be improved either by modifying the concentration of the active ingredient in the formulation or the dose frequency during treatment.

Keywords CUTANEOUS LEISHMANIASIS, QUINOLINE ANALOGS, *In vitro* ASSAYS, *In vivo* MODELS

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P2-045: *IN VITRO* CHARACTERIZATION OF A SERIES OF 1,4-DIARYL-PYRAZOLO-PYRIDINONES AND PYRAZOLO[5,1-C][1,2,4]TRIAZINES AGAINST *Leishmania donovani*

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The high toxicity associated with the current anti-leishmanial therapies, emergence of resistance, and the absence of a vaccine that is safe and effective in humans, demand the identification of new anti-leishmanials. Previously, we utilized fluorometric high-throughput screening of the ChemBridge DIVER-set™ library cassette #5 small molecule library against axenic *L. donovani* amastigotes constitutively expressing the red fluorescent protein mCherry. Of the 109 molecular scaffolds identified that exhibited an effectiveness greater than 80% of the 50μM miltefosine response, two exhibited potent antileishmanial activity: 1,4-diaryl-pyrazolo-pyridinone (1,4-DAPP) and pyrazolo[5,1-c][1,2,4]triazine (PTZ). Here, 55 analogs of these compounds were synthesized with substitution patterns aimed at increasing aqueous solubility and impart desirable pharmacokinetic properties. Host cell toxicity of human THP-1 macrophages and effectiveness of analogs against axenic and *bone fide* intracellular *Leishmania donovani* mCherry parasites was determined. Finally, an *in vivo* murine model of cutaneous leishmaniasis (CL) was used to test lead compounds. Nineteen of the 29 1,4-DAPPs and all 28 PTZs assessed exhibited half-maximal inhibitory concentration (IC_{50}) values between 0.5-10μM against axenic *L. donovani* amastigotes with selectivity indices (SI) ranging from 1 to 84. Nineteen of the 1,4-DAPPs and 16 of the PTZs exhibited IC_{50} values below 10μM against intracellular *L. donovani* amastigotes with SIs ranging from 0.43 to 25.25. The PTZ scaffold exhibited more cytotoxic events but still retained substantial potency against *L. donovani* amastigotes. Two 1,4-DAPP compounds synthesized without a methyl *ortho*- to the

pyrazolo-group to decrease potential metabolism were consistently potent against both axenic and intracellular amastigotes and non-toxic to THP-1 macrophages. In addition, these compounds reduced lesion size and parasite burden similar to the antimony control in a BALB/c-*L. major* CL model. In total, 25 compounds exhibited potent efficacy with IC_{50} values less than 5μM against intracellular amastigotes with SI values between 12.17 and 25.25, making them worthy for further study, including pharmacokinetic analysis and *in vivo* efficacy models. Utilization of this robust screening platform allows for identification of novel anti-leishmanial compounds that are both potent and selective, with efficacy in an *in vivo* CL model.

Keywords LEISHMANIASIS; DRUG DISCOVERY; MURINE MODEL

P2-047: EXPANDING THE ARSENAL AGAINST LEISHMANIASIS: NOVEL TAMOXIFEN/CLEMASTINE CHIMERA AS POTENTIAL ANTILEISHMANIALS

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The range of drugs available to treat leishmaniasis are far from ideal: they induce lethal side effects, require special infrastructure due to parenteral administration and some of them have been showing a decrease on responsiveness to treatment. Collectively, these shortcomings make the discovery of new alternative treatments an urgent matter. the search for new drugs is expensive, laborious, time-consuming and risky. Therefore, replacing an existing approved medication is an attractive strategy to accelerate drug discovery. In this context, Dr. Uliana's group has identified tamoxifen, a selective estrogen receptor modulator (SERM) and known anti-breast cancer drug, as a potent anti-leishmanial molecule, displaying one-digit micromolar activity against intramacrophage amastigotes of *Leishmania braziliensis* (EC_{50} = 1.9 µM), *L. amazonensis* (EC_{50} = 4.5 µM) and *L. chagasi* (EC_{50} = 2.4 µM), and cleared infections in all animal models when administered intraperitoneally. Clemastine fumarate, an over-the-counter first-generation antihistamine drug, has also been described by Prof. Steel's group of having submicromolar activity against intramacrophage amastigotes of *L. amazonensis* (EC_{50} = 0.40 µM) as well as equivalent activity to glucantime in a mouse model infected by this species. Both molecules have been proposed to target the same enzyme, inositol phosphorylceramide synthase (IPCS), an essential enzyme to sphingolipids biosynthetic pathway – but also exhibit other pharmacologies. To explore

this in greater detail and develop more effective selective compounds, we have built a library of tamoxifen/clemastine hybrids based on the common chemical features shared by these molecules, such as the diaryl system and the aminoalkoxy chain. Once synthesised, 35 molecules were primarily screened against *L. major* promastigotes by resazurin-based assay, which allowed for the calculation of EC_{50}. Those with an EC_{50} < 2 µM (14 compounds) were selected for further testing, such as screening against *L. amazonensis* as well as cytotoxicity evaluation in human cells, used for the calculation of the selectivity index (SI). 8 compounds showed EC_{50} < 2 µM against both species of promastigotes together with SI > 10 and are currently being tested against intracellular amastigotes, which is a more clinically relevant model of the parasite. 6 of these compounds showed submicromolar activity against at least one of the promastigotes species. The design and synthesis of this library allowed us to have some insights on the chemical features that are essential for the activity. To investigate further the mechanism of action of the hybrid molecules, current efforts are focused on turning the most active compound into a chemical probe.

P2-048: ANALYSIS OF LEISHMANICIDAL AND IMMUNOMODULATORY ACTIVITY OF NATURAL AND SYNTHETIC PRODUCTS

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Leishmaniasis designate a group of clinical manifestations caused by the infection with *Leishmania sp.* parasite that drastically affect people and animals. The treatments have several adversities such laborious administration, side effects, resistant strains, high cost and long treatment duration. In this scenario, the search for alternative treatments for Leishmaniasis that actively eliminate the parasite and promote a satisfactory immunological response in the host is necessary. The use of essential oils (OE) has been growing significantly since they contain molecules that can have several pharmacological activities which can be a source of molecular structures that can be used for synthetic substances. Here, we analysed the leishmanicidal activity and immunomodulatory activity of Guaiol, a sesquiterpene found in the OE of *Aloysia gratissima* with the advantage of being commercially acquired and others analogues synthetics of piperine (AF1 and AF2). Promastigotes of *Leishmania amazonensis (L.a)* one of the etiological agents of cutaneous and diffuse cutaneous leishmaniasis in the New World was submitted a treatment with different concentrations of the compounds and parasite viability was assessed by XTT assay in order to obtain an inhibitory concentration (IC_{50}).

Posteriorly, we verified compounds safety using the murine monocity P388D1 cell line to ensure cellular viability in the presence of the compound. Finally, the immunomodulatory activity of the compounds was also assessed by quantifying key cytokine generation (IL10 and IL12) and pattern recognition receptors (Toll like receptors membrane TLR4 and the intracellular TLR9) by real time PCR. The results demonstrate that the compounds AF1, AF2 and Guaiol was toxic for L.a. promastigote with a IC_{50} of 17.38 µM, 8.65 µM and IC_{50} 25.07 µM respectively. At 100 µM and 60 µM the compounds proved to be safe for P388D1 cell line, as can be observe a cell viability above 70%. Preliminary real time PCR results showed that cells not stimulated and treated with Guaiol at 60 µM significantly increase their expression of IL-1β. In contrast, the cells not stimulated and treated with AF1 at 60 µM reduce their expression of TLR4. Together our results imply that the compounds can modulate the immune profile of the P388D1 cells.

Keywords *Leishmania amazonensis;* TREATMENT; GUAIOL; SYNTHETIC PRODUCTS; IMMUNOMODULATORY ACTIVITY

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P2-049: LEISHMANICIDAL ACTIVITY OF PEPTIDES FROM SKIN SECRETIONS OF ECUADORIAN FROGS

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Cutaneous leishmaniasis is a neglected tropical disease that urgently requires new chemotherapeutic agents since the currently available drugs are parenteral administered and induce several side effects. The antimicrobial activity of peptides derived from the skin secretions of frogs have been recently tested in different kind of pathogens and demonstrated promissory results. Here, we have tested the leishmanicidal activity against *Leishmania (L.) mexicana* of 4 peptides derived from the skin secretions of 3 Ecuadorian frog species: *Cruziohyla calcarifer* (crusioseptins CZS-1 and CZS-4), *Agalychnis spurrelli*, (dermaseptin DRS-SP2), and *Boana picturata* (pictuseptin PTS-1). The peptides were not collected directly form the animals, but synthetized *in vitro*. The peptides were synthesized in solid phase using an automatic synthesizer (Liberty Blue, CEM), and subsequently purified by flash chromatography and HPLC until >95% purity was obtained. The identity of the peptides was confirmed with MALDI-TOF MS mass spectrometry (Axima Confidence, Shimadzu). Finally, the peptides were preserved by lyophilization. Promastigotes of *L. mexicana* and the

mammalian cell line RAW 264.7 (derived from murine macrophages) were cultivated in SDM and DMEM media, respectively. Both media were supplemented with 10% FBS and 1% antibiotics mixture (penicillin/streptomycin). The leishmanicidal and cytotoxic activities were tested using the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT salt) into formazan. All four peptides were capable to kill *L. mexicana* promastigotes in a dose-dependent manner (IC_{50} = 0.543 µM cruzioseptin-1, 0.088 µM cruzioseptin-4, 0.609 µM dermaseptin SP-2 µM and 0.100 µM pictuseptin-1. Also, all the peptides demonstrated cytotoxic activity against RAW 264.7 cells (CC_{50} = 1.113, 3.633, 1.579 and 0.8753 µM, respectively). Only cruzioseptin-4 showed a good selectivity index >10 (SI = 41.36). This peptide may be considered as a potential candidate for the drug discovery pipeline against cutaneous leishmaniasis.

Keywords *Leishmania mexicana*; CUTANEOUS LEISHMANIASIS; SKIN FROG PEPTIDES; ANTIMICROBIAL PEPTIDES

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P2-050: THE EFFECT OF PEPTIDES FROM *Lupinus mutabilis* ON THE GROWTH OF *Leishmania sp.*

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Leishmaniasis is a public health problem in Peru, with two important clinical forms, cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis (MCL). Chemotherapy to treat the disease currently has several disadvantages, and also generating resistance. Therefore, there is a need for the development of new antilesishmanial drugs for the treatment of this disease. In this work, peptides obtained from *Lupinus mutabilis* proteins have been evaluated and tested in *in vitro* growth experiments of promastigotes of different *Leishmania* strains. The peptides exert an inhibitory effect on 50% of parasite growth (IC50). Preliminary evaluation trials are shown where the results could suggest the effect of the peptides as a new alternative against leishmaniasis.

Keywords *Leishmania*; PROTEASES; PÉPTIDES; *Lupinus mutábilis*

P2-051: SYNTHETIC BISPYRAZOLE DERIVATIVES WITH LEISHMANICIDAL ACTIVITY AGAINST *Leishmania mexicana*

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Leishmaniasis mainly affects poor people, especially in LMIC countries and regions around the world with inadequate access to health services. Despite all the efforts made to generate new drugs or treatments for visceral leishmaniasis, cutaneous and mucocutaneous leishmaniasis have been relegated. In this sense, there is a great urgency to identify new molecules that can allow the generation of new pharmacological treatments for these clinical forms. Different drugs possess nitrogen heterocycles in their structure and one interesting example is the pyrazole derivatives which have demonstrated a variety of biological effects. For instance, edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) is a molecule with a pyrazole ring that is currently for the treatment of amyotrophic lateral sclerosis and has a free radical scavenger activity. Edaravone derivatives, such as 4,4'-(arylmethylene) bis(1H-pyrazol-5-ols), have antimicrobial, anti-inflammatory and anticancer bioactivities. Nevertheless, the leishmanicidal bioactivity of these heterocycles have never been reported, although similar molecules, like biscoumarins, diindolylmethanes, and 3′-(arilmetilen)-bis-(2-hydroxynaphthalen-1,4-diones) have showed strong leishmanicidal activity. Therefore, 25 derivatives (named 2a to 2y) of 4,4'-(arylmethylene)bis(1H-pyrazol-5-ols) were synthesized and the leishmanicidal and cytotoxic activities were evaluated. The bispyrazole derivatives were prepared following the Michael addition of an aromatic aldehyde to an arylpyrazolone, which is obtained by the Knoevenagel

reaction. Promastigotes of *Leishmania (L.) mexicana* were cultivated in USHMARU biphasic medium (rabbit blood agar plus SDM (Gibco, Invitrogen) supplemented with 10% FBS (Eurobio) at 27°C). For experiments, the promastigotes were changed to a monophasic SDM medium. Murine macrophages (cell line RAW 264.7, ATCC® TIB-71™) and the human hepatic cell line HepG2 (ATCC® HB-8065™) were grown in DMEM and MEM (Gibco, Invitrogen), respectively. The DMEM was supplemented with 10% FBS (Eurobio) and 100 IU/mL penicillin + 100 µg/mL streptomycin (Gibco). Both mammalian cell lines were maintained in an incubator at 37°C and 5% CO_2 atmosphere. The MTT reduction viability assay was used to evaluate the leishmanicidal and cytotoxic bioactivities and was performed by triplicate with three technical replicates for each treatment and controls. For the assays, amphotericin B and saponin were used as controls for the leishmanicidal and cytotoxic assays, respectively. All compounds were dissolved in 100% DMSO. Seven out of the 25 compounds (2k, 2n, 2r, 2s, 2v, 2w, and 2x) showed an IC_{50} below 10 µM and 14 compounds (2a, 2b, 2c, 2d, 2f, 2h, 2i, 2j, 2l, 2m, 2p, 2q, 2t, and 2u) demonstrated an IC_{50} between 10 to 32 µM against the *L. mexicana* promastigote stage. Only four compounds (2e, 2g, 2o, and 2y) had an IC_{50} above 50 µM. The cytotoxic effect (CC_{50}) in both mammalian cell lines were above 50 µM for all the compounds, except for three compounds, 2p, 2s, and 2x, that showed a CC_{50} of 43±6, 49±15, and 34±5 µM, respectively only in the RAW 264.7 cell line. Interesting, the pyrazole rings with electron withdrawing groups turned out more active than those with electron-donating groups. These promissory results must be confirmed in the amastigote stage of the parasite and then tested *in vivo* in an animal model in order to continue in the drug discovery pipeline.

Keywords *Leishmania mexicana*; CUTANEOUS LEISHMANIASIS; BISPYRAZOLE DERIVATIVES; NITROGEN HETEROCYCLES

Financing Research grant by Universidad UTE, research grant by COCIBA-USFQ

P2-052: ANTILEISHMANIAL AND IMMUNOMODULATORY EFFECTS OF TRITERPENOIDS COMPOUNDS: AN APPROACH TO ACTION MODE BY *IN VITRO* AND *IN VIVO* ASSAYS

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Cutaneous leishmaniasis (CL) is the most frequent clinical form of leishmaniasis, a disease that affects the population of the tropical and sub-tropical regions of the world. This pathology produces ulcerous lesions on exposed body parts. The pharmacological treatment presents certain complications due to its form of administration, adverse effects, and increased resistance to treatment. Thereby, the use of novel molecules—natural and synthetic—has become a therapeutic alternative for the treatment of this disease. In this study, 10 structurally similar compounds, classified as limonoid-, pentacyclic-, saponin-, and lupine-type, were identified through *silico* and *in vitro* studies using 11α,19β-dihydroxy-7-acetoxy-7-deoxoichangin as the template. The physicochemical characteristics and activity against *Leishmania (Viannia) panamensis* of these 10 compounds were determined, and the therapeutic potential of the active compounds was validated in *in vivo* studies performed in golden hamsters with experimental CL. The three most active triterpenoid compounds were, from the pentacyclic subgroup, 18β-glycyrrhetinic acid (**2**), oleic acid (**3**), for which antileishmanial activity was evidenced by their values of median effective concentration (EC_{50}; 70.9 ± 12.2 μM and 28.7 ± 3.8 μM, respectively) and ammonium glycyrrhizin from the saponin subgroup (**4**), with an EC_{50} of 50.7 ± 3.8 μM. These compounds selectively affected the intracellular form (amastigote) of *L. (V.) panamensis*, while presenting low toxicity against the host cells. Moreover, compound **2**

induced an increase the production of nitric oxide via NF-kB as well as the presence of lipid vacuoles in infected human macrophages. The *in vivo* assays showed that 20%–80% of the infected hamsters treated with compounds **2–4** showed clinical improvement, which indicates that these synthetic compounds serve as a therapeutic strategy for the control and management of the cutaneous disease caused by *Leishmania (V.) panamensis*.

Keywords LEISHMANIASIS; TREATMENT; IMMUNOMODULATION; *IN VIVO;* TRITERPENOIDS

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P3-038: A NEW INFECTION MODEL FOR *Leishmania donovani* BY USING HUMAN IPS DERIVED MACROPHAGES

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Visceral leishmaniasis is a deadly infectious disease claiming up to 40 000 lives each year. Despite its huge burden, it is still a neglected disease. There is an urgent need for new drugs against this infection, but the intracellular lifecycle of *Leishmania* complicates the search for new drug candidates. Currently, we are testing the antileishmanial activity of compounds against intracellular amastigote *Leishmania donovani* using primary mouse peritoneal macrophages as host cells. Now we propose a new infection model where induced pluripotent stem cell (iPS) derived macrophages (iMAC) replace the mouse macrophages. Human iPS cells were differentiated to macrophages using embryoid body (EB) differentiation. We infected these with *L. donovani* promastigotes and determined the infection rate and cell survival using a DAPI/Alexa Fluor 488 Phalloidin staining followed by high content imaging. We optimized the seeding density of the iMAC and the multiplicity of infection (MOI) and concluded that the age of the EB-culture does not influence the infection process. With these optimized conditions, we reach infection rates over 60%. Currently, we are performing antileishmanial efficacy testing with reference drugs and we will compare the results obtained with iMAC to those obtained with primary mouse macrophages. Once established, this new model will accelerate antileishmanial drug discovery by providing human host cells in large number without the use of animals.

Keywords INDUCED PLURIPOTENT STEM CELL; *Leishmania donovani*; DRUG DISCOVERY; IN VITRO ASSAY

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P3-039: EFFICACY OF TOPICAL MILTEFOSINE FORMULATIONS IN AN EXPERIMENTAL MODEL OF CUTANEOUS LEISHMANIASIS

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Cutaneous leishmaniasis (CL) is a neglected tropical disease endemic in ~ 90 countries, with an increasing incidence. Presently available pharmacotherapy implies the systemic administration of moderately/very toxic drugs. Miltefosine (Milt) is the only FDA-approved drug to treat CL via the oral route (Impavido®). It produces side effects; in particular, teratogenic effects are of concern. A topical treatment would have the great advantage of minimising the systemic circulation of the drug, preventing side effects. We prepared dispersions containing Milt and liposomes of different compositions to enhance/modulate trans-epidermal penetration and evaluated *in vitro* and *in vivo* efficacy and toxicity, *in vitro* release rate of the drug and particles size stability with time. Treatments were topically administered to BALB/c mice infected with *Leishmania (Leishmania) amazonensis*. The dispersions containing 0.5% Milt eliminated 99% of the parasites and cured the lesions with a complete reepithelisation, no visible scar and re-growth of hair. Fluid liposomes decreased the time to heal the lesion and the time needed to eliminate viable amastigotes from the lesion site. Relapse of the infection was not found 1 month after treatment in any case. Ultraflexible liposomes on the other hand had no significant *in vitro* effect but decreased *in vivo* efficacy. A topical Milt formulation including fluid liposomes seems a promising treatment against CL.

Keywords CUTANEOUS LEISHMANIASIS; MILTEFOSINE; LIPOSOMES; TOPICAL TREATMENT

Financing Fundación Bunge y Born; Secr. Ciencia y Tecnología – Universidad Nacional de Córdoba; CONICET

P3-040: UTILITY OF A BIOLUMINESCENT SYSTEM BASED ON THE LUCIFERASE REPORTER GENE FOR THE STUDY OF ENERGY METABOLISM IN *Leishmania* SPECIES.

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In the search for new leishmanicidal drugs with greater efficacy and lower toxicity than those currently available, reporter genes represent a good tool for the generation of efficient and reliable biological models. The luciferase reporter gene uses ATP as a substrate to generate luminescence, being an indicator of the effect of leishmanicidal activity on energy metabolism. The usefulness of the luciferase reporter gene has been proven in Old World *Leishmania* species; nevertheless, the high variability among species makes it necessary to evaluate the utility of this system in species present in America, and define if transfection alters the biological behavior of new world *Leishmania* species. The pLEXSY hyg2.1 plasmid, amplified in *E. coli* Dh5α, was linearized by enzymatic digestion and introduced into promastigotes by electroporation. The selection was performed with hygromycin at the median lethal concentration (LC_{50}) for each of the *L. panamensis, L. braziliensis,* and *L. infantum* strains. Gene insertion was confirmed using DMNPE as substrate by quantifying luminescence. Naphthoquinone and Triton X100 were used as controls for ATP loss. The median infective concentration (IC_{50}) in U937 macrophages as well as the sensitivity of wild-type and transfected strains to commonly used leishmanicidal drugs were determined by light microscopy. All strains integrated the plasmid. The degree of luminescence observed suggests that *L. panamensis* and *L. braziliensis* produce more copies of the gene, while *L. infantum* produces less luminescence, a fact that may be ascribed to the differences in metabolism that occur between different species.

Luminescence assays indicate that the model allows detecting ATP loss and associating whether it is due to alterations in the cytoplasmic membrane or mitochondrial damage. Transfection did not alter the infectivity or growth kinetics of wild-type and transfected strains. Drug sensitivity was not altered, except for pentamidine in promastigotes of *L. panamensis* and amastigotes of *L. braziliensis,* where greater sensitivity was observed in wild strains. Transfection *in L. braziliensis, L panamensis,* and *L. infantum* allowed the establishment of a model for the study of energy metabolism in *Leishmania* promastigotes, without negative alterations in infectivity and sensitivity, which will be useful to determine whether new leishmanicidal compounds affect mitochondrial function.

Keywords *Leishmania braziliensis*; *L. panamensis*; *L. infantum*; LUCIFERASE; METABOLISM

P3-041: ASSOCIATION OF ORAL AND TOPICAL DRUGS IN THE TREATMENT OF CUTANEOUS LEISHMANIASIS CAUSED BY *Leishmania (viannia) braziliensis* IN BALB/C MICE.

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The search for treatment of ATL with drugs of easy administration, low toxicity and with greater therapeutic efficacy is the object of studies aimed at curing the disease. The lack of response in some cases and the adverse effects generated the need to use second line drugs, drug repositioning, topical therapies, and drug combinations in order to increase the effectiveness of the treatment. Given this scenario, the study evaluated the efficacy, adverse effects and immunomodulatory action of the association of miltefosine (M) with oral pentoxifylline (P) and photodynamic therapy with *Magonia pubescens* hydrogel loaded with Carbon dots (PDT) in the treatment of cutaneous leishmaniasis in BALB/c mice infected with *L. (V.) braziliensis*. For this, an experimental in vivo study was carried out, in which 54 female mice of the *Mus musculus* species were divided into nine groups, and treated with the following therapies and associations: M, P, PDT, M+P, P+PDT, M+TFD and MPPDT. Efficacy evaluation criteria were: culture, amastigote presence, serial dilution and MTT, quantification of parasites per paw using the Real Time Polymerase Chair Reaction (qPCR), measurement

of paws, animal weighing, biochemical and immunological exams. Statistical analyzes were performed using the R Studio program and Prisma 7 (Kruskal-Wallis test, followed by Dunn's method), and statistical significance was defined by a p-value <0.05 and a confidence interval of 95%. The analyzes showed that the association of the three therapies (M+P+PDT) was more satisfactory. The association P+PDT proved to be more effective than treatment with P alone, and statistically similar to treatment with PDT. P alone showed similar results to the CP group. PDT appears to be more effective than pentoxifylline treatment. The qPCR proved to be efficient to quantify the number of parasites in this experimental study. Regarding the production of nitric oxide, miltefosine alone influenced the decrease in macrophage production, and the P+PDT association showed significant relevance (p=0.0245) and immunomodulatory influence in the treatment of BALB/c mice infected with *L. braziliensis*. However, the importance of the association of drugs and new methodologies is emphasized, since some results can become a treatment option. However, future work is needed, using a larger number of animals because there was unexpected death of mice.

Keywords LEISHMANIASIS, CUTANEOUS; PHOTODYNAMIC THERAPY; CARBON DOTS; DIAGNOSIS; *Pubescens magonia*

P3-043: NANOASSEMBLIES MADE FROM AMPHIPHILIC ANTIMONY(V) COMPLEXES TARGET INFECTION SITES IN THE *Leishmania donovani* EXPERIMENTAL MODEL

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It has been reported previously that amphiphilic antimony(V) complexes obtained from reaction of $KSb(OH)_6$ with octanoyl-N-methylglucamide (SbL8) or decanoyl-N-methylglucamide (SbL10) are active by oral route in murine models of visceral and cutaneous leishmaniases caused by New World *Leishmania* species. These complexes self-assemble in aqueous solution, forming micelle-like nanoparticles, however, the ability of these nanosystems to carry and deliver Sb and other drugs to infected tissues and cells has not been fully elucidated. The present study aims to test the hypothesis that Sb nanoassemblies may favor drug targeting of infection sites. First, the kinetic stability of SbL8 and SbL10 nanoassemblies was evaluated upon dilution in media of different pHs, using a fluorescent lipophilic marker. Secondly, we evaluated the Sb uptake by macrophages (THP1 cells) and liver after parenteral administration to mice, comparing the amphiphilic Sb complexes to Glucantime®. Thirdly, the *in vitro* and *in vivo* antileishmanial activities of SbL8 and SbL10 was compared to Glucantime® in models of visceral leishmaniasis (VL) caused by *Leishmania donovani*. The nanoassemblies were found to be stable at neutral pH, but suffered conformational change and released incorporated lipophilic substance upon acidification of the media at pH values close to that of the

gastric fluid and parasitophorous vacuole. The amphiphilic complexes promoted much greater accumulation of Sb in THP1 cells, when compared to Glucantime®. The hepatic accumulation of Sb was also much greater from the amphiphilic complexes, when compared to Glucantime®, after parenteral administration to mice. SbL8 and SbL10 were found to be more active against intracellular amastigotes than axenic amastigotes, the difference being much more pronounced for SbL10. SbL10 was also much more active than Glucantime® and less cytotoxic than SbL8 against macrophage cell line, resulting in selectivity index as high as 800. BALB/c mice were treated for 20 days (daily) with SbL8 and SbL10 by intraperitoneal (IP) and oral routes. The amphiphilic complexes at 20 mg Sb/kg/d by IP route showed greater antileishmanial activity than the same complexes given orally at 200 Sb/kg/d and were as effective as the standard drug Glucantime® at 200 mg Sb/kg/d IP. SbL8 and SbL10 were equally effective in reducing parasite loads in both the liver and spleen. Our data is consistent with a high drug targeting of the liver after parenteral administration. This work supports the ability of Sb nanoassemblies to carry lipophilic drugs in biological media at neutral pH and to deliver Sb and other incorporated lipophilic drugs to infected cells and tissues.

Keywords ANTIMONY; AMPHIPHILIC; MICELLE-LIKE NANOPARTICLES; VISCERAL LEISHMANIASIS

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P3-046: MSC THERAPY IN THE TREATMENT OF EXPERIMENTAL CUTANEOUS LEISHMANIASIS: COMBINATION WITH CHEMOTHERAPEUTICS AND EVALUATION OF EXTRACELLULAR VESICLES

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Leishmaniasis is a neglected disease caused by *Leishmania spp*.. One of its characteristics is an imbalance of host immune responses to foster parasite survival. In this setting, mesenchymal stromal cells (MSCs) may be a viable therapeutic alternative, given their well-established immunomodulatory potential. In addition, extracellular vesicles produced by MSCs (MSC-EVs) have already been shown to have similar capabilities as the cells that originated them. In this work, we compared the effects of therapy with bone marrow (BM)- and adipose tissue (AD)-derived MSCs in leishmaniasis caused by *Leishmania amazonensis* in C57BL/6 mice. After determining the most effective MSC source, we then combined these cells with meglumine antimoniate (a pentavalent antimonial commonly used for the treatment of leishmaniasis) to treat the infected mice and evaluated the ability of MSC-EVs treatment of these cells to promote the same effects as treatment using the MSCs. In the *in vitro* experiments, co-culture of AD-MSCs and BM-MSCs with *Leishmania amazonensis*-infected macrophages was performed to understand the influence of both MSC sources in infected cells. While in the *in vivo* experiments, infected C57BL/6 mice were treated with AD-MSCs and BM-MSCs to evaluate the capacity of these treatments to promote protection against the *L. amazonensis* infection. After this, we combined MSCs from the most effective source with meglumine antimoniate to assess whether this combination would improve treatment with just MSCs. At last, we used the

MSC-EVs from the most effectiv cells were used to treat *L. amazonensis*-infected C57BL/6 mice. As results, in the *in vitro* infection, co-culture of *Leishmania amazonensis*-infected macrophages with BM-MSCs, compared to AD-MSCs, led to a higher parasite load and lower production of nitric oxide. Fibroblasts grown in conditioned medium from co-cultures with AD-MSCs promoted faster wound healing. Despite a non-significant difference in the production of vascular endothelial growth factor, we observed higher production of tumor necrosis factor-α and interleukin (IL)-10 in the co-culture with AD-MSCs. *In vivo*, treatment of infected mice with BM-MSCs did not lead to disease control; however, the use of AD-MSCs was associated with partial control of lesion development, without significant differences in the parasite load. AD-MSCs combined with meglumine antimoniate reduced lesion size and parasite load when compared to PBS and AD-MSC groups. At the infection site, we detected a small production of IL-10, but we were unable to detect production of either IL-4 or Interferon-γ, indicating resolution of infection without effect on the percentage of regulatory T cells. Treatment with AD-MSC-EVs did not (partially) control the lesion in the acute phase of infection, as is seen in treatment with AD-MSCs, however, the lesion in EVs-treated mice was smaller during the chronic phase of infection, while the load, on the other hand, did not present significant differences between the groups. The combined treatment for cutaneous leishmaniasis with AD-MSCs and meglumine antimoniate may be a viable alternative to existing treatments and the use of extracellular vesicles instead of the MSCs themselves seems promising, but needs to be further evaluated.

Keywords MSC; *Leishmania*; CELL THERAPY; VESICLES; STEM CELL

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P3-046.1: SMALL MOLECULES TARGETING *Leishmania braziliensis*: POTENTIAL TARGETS FOR CHEMOTHERAPY

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Cutaneous Leishmaniasis (CL) caused by *L. braziliensis* presents as several clinical forms, which range from a localized ulcerated lesion to disfiguring lesions in mucosal areas. *L. braziliensis* can also cause disseminated leishmaniasis, a severe form of disease that frequently presents with mucosal involvement. CL affects 1.5 million people worldwide, and the current first line treatment are pentavalent antimony compounds that present toxicity and are subject to parasite resistance, making it evident the need for better therapeutical options. One of the challenges in the development of novel antileishmanial compounds is achieving potent activity against the intracellular stage of the parasite, the stage present in the mammalian host, without harming the host cell. Previously, we identified a compound series that displayed effective antiparasitic activity against *L. braziliensis*. Herein, we explored these compounds and evaluated their effectiveness employing murine macrophages, followed up by experiments *in vivo*. Macrophages infected with *L. braziliensis* and exposed to the compound series in a dose dependent manner showed that molecules Cpd1 and Cpd2 reduced the percentage of infected cells and the number of intracellular amastigotes in a significant manner. Similar results were obtained upon infection with *L. major* and both compounds also did not

exhibit cellular toxicity. Parasite killing was accompanied by an increase in the production of TNF and superoxide and both molecules are associated macrophage effector functions. Lastly, in a pre-clinical mouse model of CL caused by *L. braziliensis*, we observed that topical application of Cpd1, in gel-based form employing bacterial cellulose, impaired lesion development and significantly reduced parasite burden. These results indicate that this compound series can be further explored for the development of novel chemotherapeutic alternatives for CL caused by *L. braziliensis,* the causative agent of localized, mucosal and disseminated leishmaniasis.

Keywords TREATMENT; CHEMOTHERAPY; BIOCURATIVE

P4-011: BACTERIAL CELLULOSE BIOCURATIVES FOR THE TOPICAL TREATMENT OF CUTANEOUS LEISHMANIASIS

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In Brazil, cutaneous leishmaniasis (CL) is mainly caused by *Leishmania braziliensis*. Pentavalent antimonials (Sb^v) remain the first-line drug on treatment for CL despite the limitations regarding toxicity and increasing reports of therapeutic failure. Therefore, the search for alternative options for treatment that are safe, efficient and of easy application remains necessary. We have show that DETC, a SOD1 inhibitior, in association with a bacterial cellulose (BC) biocurative, reduced parasite burden and inhibited lesion development in a pre clinical model of CL caused by *L. braziliensis*. We thus hypothesized that BC biocuratives in association with DETC (BC-DETC) could act in conjuction with pentavalent antimonials to reduce the burden of disease in CL patients. To this end, we performed physicochemical characterization of BC-DETC employing scanning electron microscopy (SEM) and x-ray diffraction (XRD). In addition, we performed an *in vitro* release assay by spectrophotometry and evaluated the stability of DETC onto BC by spectrophotometry and thermogravimetry. SEM images of BC-DETC showed DETC aggregates across the entire surface. The absence of crystallographic peaks, seen by XRD analysis, indicated that DETC was succesfully incorporated onto BC biocuratives. In vitro release experiments showed a accumulative mass release of 22% and 14%, at 5 minutes and 24 hours, respectively, indicating possible degradation of DETC. Thermogravimetry analysis complemented our findings that strongly indicating that DETC is not stable when incorporated onto BC. Despite our

results showing that DETC is short lived when incorporated onto BC, as suggested by degradation experiments, we performed an initial a pilot, proof-of-concept trial, to evaluate the efficacy of topical application of BC in CL patients. A total of 20 patients were randomized in two groups assigned to receive either parenteral Sb^v alone or parenteral Sb^v plus topically applied BC bio-curatives. CL patients treated with Sb^v + topical BC bio-curatives had a significantly higher cure rate at 60 days post initiation of treatment compared to CL patients treated with Sb^v alone (P=0.01). At day 90 post initiation of treatment, cure rate was similar in the two groups as was overall healing time. Adverse effects or local reactions to topical BC application were not observed. This pilot trial shows that the potential use of a combined therapy consisting of topical BC bio-curatives and parenteral Sb^v in favoring healing of CL lesions caused by *L. braziliensis*, at an early time point.

Keywords CHEMOTHERAPY; TOPICAL TREATMENT; BIODRESSING

P4-012: DEVELOPMENT OF MICROPARTICULATED IMPLANTS BY SPRAY DRYING FOR SUSTAINED RELEASE OF ANTILEISHMANIAL DRUGS

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Local cutaneous leishmaniasis (LCL) therapy is based on multiple injections with drugs that can cause severe systemic toxicity. Pain, unwellness, and the required frequent visits to distant hospitals are obstacles for treatment completion. Since topical creams have not demonstrated adequate drug absorption and effectiveness, we envisaged to develop novel drug formulations that allow a single local injection to be effective. For that, using spray drying technique with a three-fluid nozzle set, which allows a shell-core structure, we produced biodegradable PLGA (poly(lactide-co-glycolide acid) microparticles blended with PLA or PVP containing amphotericin B (AmB) in the core, as a prototype formulation for the treatment of LCL in a sustained drug release fashion. The fabrication process showed 60-70% yield; average particle size of 13,5 µm; zeta potential of -12,6 mV, and >70% drug incorporation rate. MEV images showed spherical and rough particle topology. *In vitro* drug release kinetics showed that PLGA/AmB microparticles promoted much slower release than free AmB within 48 hours. Cytotoxicity studies using murine BMDM cells showed that PLGA/AmB and PLGA microparticles were non-toxic to mammalian cells. Histopathological studies in mice showed that PLGA/AmB particles injected s.c. were effectively taken up by dermal macrophages. BALB/c mice infected in the ear pina with *Leishmania amazonensis*-GFP were given a single s.c. injection with PLGA/AmB or free AmB in deoxycholate (Anforicin®).

PLGA/AmB-treated lesions significantly controlled lesion growth and parasite burden as compared with free AmB. This study shows the effectiveness of spray drying fabrication of microparticulated PLGA/AmB implants in the treatment of local cutaneous leishmaniasis, and their potential applicability with newly discovered antileishmanial drugs.

Keywords CUTANEOUS LEISHMANIASIS; CHEMOTHERAPY; AMPHOTERICIN B; POLYMERIC MICROPARTICLE; SPRAY DRYING

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P4-014: STEROL REMODELING BY PHARMACOLOGICAL ACTION AND POTENTIAL THERAPEUTIC TARGETS BY THE SERINE PROTEASE PATHWAY USING A SUBTILISIN INHIBITOR

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Subtilisins (SUB) found in all organisms, are enzymes important in the post-translational steps of protein processing. Studies has been described that SUB are essential to *Leishmania*. Transcription factors such SREBP have been established as lipid synthesis transcription factors in mammalian and fungi, especially for cholesterol and fatty acid synthesis, and its factor is regulated by SUB. In *Leishmania s*p, no transcription factor was already demonstrated. The ergosterol biosynthesis pathway has been exploited as a pharmacological target. Trypanosomatids produce ergosterol and other sterols; sterols are methylated by SMT in one of the final steps of the sterol biosynthesis pathway (SBP) but this reaction does not occur in mammalian cells due to the absence of SMT. Although, available information about the mechanisms of the regulation and remodeling of sterol-related genes is scarce. In this context, we investigated compensatory mechanisms of the SBP using an inhibitor of HMG-CoA and by developing drug-resistant parasites to evaluate (sterol remodeling, cross-resistance and gene expression)as well as we evaluated potential therapeutic targets mediated by lipid way and exploring the localization and the SUB play role on *L. amazonensis*. Simvastatin-resistant *L. amazonensis* parasites (LaSimR)underwent reprogramming of sterol metabolism manifested as an increase in cholestane- and stigmastane-based sterols and a decrease in ergostane-based sterols. The levels of SMT, sterol C14-α-demethylase, and SUB were increased in LaSimR. LaSimR was cross-resistance to ketoconazole (C14DMi)and remained sensitive to terbinafine. Sensitivity of the LaSimR to other antileishmanial drugs unrelated to the SBP, such as trivalent antimony and pentamidine, was similar to that of the WT; LaSimR was cross-resistant to miltefosine, serine protease inhibitor(SPi)TPCK, subtilisin-specific inhibitor PF-429242, and tunicamycin. *Leishmania* proved to be more sensitive to SPi, thus we chose to carry out more tests using just PF-429242, which is an inhibitor of human S1P and in addition to already having tests demonstrating its effect on some microorganisms. Using catalytic domain antibody to SUB we performed TEM and FACS, which demonstrated SUB has broad localization throughout the cytoplasm and membrane of promastigote form with foci in the flagellar pocket. In silico,

the similarity between SUB of different *Leishmania* species and that of the human was determined and based on molecular docking, we evaluated the interaction capacity of a SPI against both life cycle forms of *Leishmania*. PF-429242 significantly inhibited the growth of promastigotes of four different strains (IC50= 3.07; 0.83; 2.02 and 5.83 µM against LTB0016, PH8, Josefa, and LV78 strains) whilst having low toxicity in the host macrophages(170.30µM). We detected by FACS, using a catalytic domain ab from SUB that there is a higher expression of SUB in amastigote; however, the PF-429242 had a low effect against this intracellular form with an IC50 of>100µM for intracellular amastigotes for the LV78 strain as axenic amastigotes (94.12µM). In conclusion, even though PF-429242 does not affect the intracellular forms, this drug will serve as a tool to explore pharmacological and potentially leishmanicidal targets. Additionally, findings on the regulation of the sterol pathway can support the development of drugs and protease inhibitors targeting this route in parasites.

Keywords SUBTILISIN; SERINE PROTEASE; STEROL PATHWAY; *Leishmania amazonensis*; PHARMACOLOGICAL TARGET

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P4-015: NOVEL METHODS TO QUANTIFY MILTEFOSINE, PAROMOMYCIN AND AMPHOTERICIN B IN SKIN BIOPSIES FROM POST-KALA-AZAR DERMAL LEISHMANIASIS PATIENTS

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Miltefosine [1], amphotericin B and paromomycin [2] are all approved drugs for the treatment of various clinical presentations of the neglected parasitic disease leishmaniasis. In cutaneous leishmaniasis and post-kala-azar dermal leishmaniasis (PKDL), *Leishmania* parasites reside and multiply in the dermis of the skin. These treatment options are currently studied in combination therapy trials for the treatment of these dermal leishmaniases. There is an urgent need for accurate assays to determine miltefosine, amphotericin B and paromomycin concentrations in human skin tissue, to assess target site pharmacokinetics of these antileishmanial drugs to enable further optimization of the dosing regimens. To date, no bioanalytical assay was available to assess human skin concentrations of these antileishmanial drugs. We here describe the development and validation of sensitive and accurate methods to homogenize human skin tissue and quantify miltefosine, amphotericin B and paromomycin in 4-mm human skin biopsies utilizing high-performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Quantification of pharmaceutical compounds in skin tissue is challenging because of low expected concentrations, small typical sample volumes, and hard nature of the skin structure itself [3]. Given that the true analyte recovery from skin tissue is difficult to assess, the extent of homogenization plays a crucial role in the quantification. We developed a novel enzymatic tissue digestion method,

suitable for the analysis of these antileishmanial drugs. The digestion method, baes on collagenase A incubation overnight (± 16 hours) at 37°C, led to completely dissolution of full thickness human skin biopsies and acceptable recovery of the drug analytes. Final extracts were injected on a Gemini C18 column for both miltefosine and amphotericin B, using alkaline eluent for separation and elution with miltefosine assays and acidic eluent for amphotericin B. Ion-pair chromatography was performed on an UPLC column for separation of paromomycin, using heptafluorobutyric acid as ion-pair reagent. Quantification was performed using a quadrupole – linear ion trap mass spectrometer. The methods were validated following FDA and EMA guidelines over linear calibration ranges of 4-1000, 10-2000, and 5-1000 ng/mL for miltefosine, amphotericin B and paromomycin, respectively. Validation parameters were all within internationally accepted criteria, including intra- and inter-assay accuracies and precisions within± 15% and ≤ 15% (within± 20% and ≤ 20% at the lower limit of quantitation). Patient human skin tissues were measured using the concentration described by the calibration curves in ng/mL. Conversion of skin tissue concentrations of the drugs was performed using the measured concentration times the added digestion solution in mL, and eventually divided by the mass of the skin tissue sample in mg to get the concentration µg/g skin. The lowest and highest concentrations measured for miltefosine, amphotericin B and paromomycin in patient skin tissue were respectively 1.7-117.9 µg/g, 11.8-2030 µg/g, and 3.4-51.5 µg/g, Using the developed methodologies human skin tissue samples were successfully quantified in skin biopsies from PKDL patients treated in India, Bangladesh and Sudan.

Keywords LC-MS/MS; PKDL; TREATMENT

P4-016: PROTOCOL FOR A RANDOMISED, OPEN LABEL MULTICENTRE, NON-INFERIORITY CLINICAL TRIAL FOR NEW TREATMENT MODALITIES FOR CUTANEOUS LEISHMANIASIS CAUSED BY *Leishmania tropica*

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Cutaneous leishmaniasis (CL) is a neglected tropical skin disease, caused by the protozoan Leishmania. Although not a fatal disease, skin lesions often develop into ulcerating, disfiguring wounds and scars causing psychosocial suffering due to stigmatisation and discrimination. In Pakistan, CL is highly endemic and Leishmania tropica is the predominant species in Balochistan and Khyber Pakhtunkhwa provinces. Since decades, the mainstay treatment for CL is with pentavalent antimonial drugs, which are given in long courses (3-6 weeks) of painful injections. This antimonial treatment is contra-indicated for various vulnerable groups (pregnant women and patients with underlying morbidities), due to the potential toxic side effects. Besides that, this treatment is scarcely available in Pakistan public hospitals, and in addition has important financial and gender barriers to access treatment. Médecins sans Frontières has five CL diagnostic and treatment centres in the

two endemic provinces. Topical thermotherapy by radiofrequency generated heat, and oral miltefosine are effective in several Leishmania species, however these treatments have limited evidence for effectiveness in CL caused by L. tropica. Thermotherapy requires a single treatment session and miltefosine is an oral treatment and can be provided at primary health care level. The combination of these two treatments could shorten the treatment duration of miltefosine and have an additive effect from their different modes of action. The study is aimed to evaluate the effectiveness and safety of the thermotherapy (ThermoMed™), miltefosine (Impavido®) and the combination of the two treatments, in two cities with high prevalence of CL caused by L. tropica. We aim to find a treatment similar or better than the standard of care with intralesional injections of antimonial treatment (Glucantime®). We will perform a randomised, open label, multicentre, non-inferiority clinical trial (RCT), evaluating the efficacy and safety of new treatment options in four study arms: 1) topical thermotherapy (ThermoMed®, radiofrequency generated heat of 50°C, 30 seconds application, one session); 2) oral miltefosine capsules (2.5 mg/kg, 28 days); 3) a combination of thermotherapy (one session) and miltefosine (21 days); and 4) compared to I usuallthe standard of care with eight sessions (bi-weekly) of intralesional injections (local into the CL lesions) with meglumine antimoniate. We will recruit 832 CL patients (208 per study arm), aged ten years or older, and have with a parasitologically confirmed CL diagnosis, in two CL treatment centres in Quetta and Peshawar, Pakistan. Primary endpoints are initial cure rate (re-epithelisation and flattening of lesions) at day 91, and severity, seriousness and frequency of adverse events by treatment group. A descriptive analysis is followed by logistic regressions to analyse possible associations between the treatment and dichotomous primary outcomes of final cure/failure. We hope to identify an affordable, safe and effective treatment for CL caused by L. tropica. If successful, it can be implemented in primary healthcare facilities and increase treatment accessibility for CL patients.

Keywords *Leishmania tropica*; MILTEFOSINE; THERMOTHERAPY; PAKISTAN

P4-017: NEW DRUG COMBINATIONS FOR THE TREATMENT OF VISCERAL LEISHMANIASIS

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Current treatments for human leishmaniasis present several problems related to the development of resistance, side effects, high cost, poor oral bioavailability, chemical instability and prolonged treatments. Therefore, in the absence of an effective vaccine, there is a need for research into new drug treatments to overcome these problems. Drug repurposing and the combination of drugs with different mechanisms of action, which allow the reduction of the drug administered, thus reducing side effects and treatment times, are valid and cost-effective approaches to incorporate new treatments for these diseases. In a recent published screening of two commercial collections of 1,769 replacement drugs using splenic explants from mice infected with a strain of *L. donovani* with infrared fluorescence[1], 42 compounds with antileishmanial activity < 1µM were selected. From these compounds, we have chosen Nifuratel (NFT), a synthetic nitrofuran whose potency and selectivity point it as a promising oral drug for the treatment of visceral leishmaniasis. In addition, NFT, administered by the intralesional route, produced complete parasitological clearance against cutaneous model of *L. major* cutaneous leishmaniasis[2]. We have made several combinations of NFT with two drugs already used in the treatment of visceral leishmaniasis, miltefosine (MTF) and paromomycin (PMM), in order to reduce the doses of both drugs and, therefore, their potential toxic effects. To this end, we exposed either axenic amastigotes (isolated from the bone marrow of infected mice) or intramacrophagic amastigotes (obtained from primary cultures of murine spleen explants) both isolated from Balb/c mice infected with an infrared strain of *L. donovani*, to combinations of NFT/MTF and NFT/PMM in proportions 1/10 to 1/60 and 1/100 to 1/300,

respectively. Combination results were analysed with the Calcusyn statistic program[3]. After observing a clear antileishmanial synergy in both combinations, in vivo experiments in a model of chronic visceral leishmaniasis were performed with the combination of NFT + MTF both by oral administration, according to the following regimen 50 mg/kg/day NFT + 10 mg/kg/day MTF for 10 consecutive days. These experiments were performed with a strain of *L. donovani*-luc that allowed us to observe the development of the infection through the IVIS Spectrum image recording system. The administration of 50 mg/kg/day NFT produced a 55% parasitic burden reduction, while the reduction after the treatment with 10 mg/kg/day MTF was estimated to be > 85%. Interestingly, the effect after combination of both drugs was slightly superior to that found for MTF (10 mg/kg/day) alone (>90 %). Therefore, further combination regimens as well as combination of NFT with PMM, are needed to determine whether the combination of both drugs can have a clear antileishmanial effect *in vivo*.

Keywords COMBINATION THERAPY; EX-VIVO SPLENIC EXPLANT PLATFORM; IN VIVO IMAGING; NIFURATEL

P4-018: SPATIOTEMPORAL ANALYSIS OF ENDEMIC AND EPIDEMIC PATTERNS IN THE OF HUMAN VISCERAL LEISHMANIASIS IN A SMALL MUNICIPALITY IN SOUTHEASTERN BRAZIL

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Human visceral leishmaniasis is a severe systemic infectious disease, with a wide geographic distribution and a high morbidity and mortality rate. It constitutes a public health problem in tropical and subtropical regions. Visceral leishmaniasis has shown endemic patterns and episodes in urban areas, however, there are still gaps in knowledge with regards to disease transmission. Objective: This study aimed to analyze the spatiotemporal dispersion of visceral leishmaniasis cases in the municipality of Araçuaí, Minas Gerais. A study spatiotemporalof confirmed visceral leishmaniasis cases was conducted. This study focuses on the disease patterns of cases notified to the Notifiable Diseases Information System from the municipality of Araçuaí. The cases were separated into two periods: 2012 to 2014, characterized as an endemic period, and 2015 to 2017, epidemic period. The incidence rate was calculated, and for spatial analysis, the kernel map, directional distribution ellipse, and space-time scanning techniques were used. The correlations between visceral leishmaniasis cases and exposure variables (precipitation, humidity, and temperature) were calculated. Between 2012 and 2017, 68 new cases of visceral leishmaniasis were reported in residents of the Araçuaí. Of these, 20 cases occurred during the

endemic period (2012–2014) and 48 occurred during the epidemic period (2015-2017). The mean incidence of visceral leishmaniasis in the endemic period was 18.5 (95% confidence interval (CI) 5.9-32.5) and 44.4 in the epidemic period (95%CI, 12.0-28.6) by 100,000 inhabitants. The relative risk for the epidemic period was 2.4 (95% CI 1.4-4.1) when compared to the endemic period. A higher incidence of the disease was observed in rural areas. Kernel mapping analysis revealed hotspots in the urban area of the municipality. The directional distribution ellipse encompasses the urban perimeter and part of the rural area of the municipality, expanding eastward during the epidemic period. Spatial analysis revealed a high-risk cluster in rural areas. A positive correlation was observed between visceral leishmaniasis cases and temperature during the endemic period. Both the prevalence and incidence depend on understanding the different forms of the disease associated with geographically isolated transmission cycles and regional differences in surveillance. With regards to the expansion, some studies also indicate that the disease may be associated with the low impact of the control measures employed, possible improvement of the diagnosis and notification system, and people's mobility. Spatial analysis allowed us to outline the epidemiological scenario of human cases of visceral leishmaniasis in the municipality during the endemic and epidemic periods. The number of visceral leishmaniasis cases in Araçuaí remains high, considering its incidence in Brazil. It is distributed in the urban and rural areas of the municipality, with expansion during the epidemic period. These results suggest that ideal conditions for establishing and maintaining transmission are found in these locations. The pattern of occurrence of visceral leishmaniasis is not static, and the disease may expand to other areas of the municipality. These findings may be useful in case surveillance and in the work of health professionals and managers as well as in guiding further research.

Keywords VISCERAL LEISHMANIASIS; SPATIO-TEMPORAL ANALYSIS; ENDEMIC AND EPIDEMIC PERIODS

P4-019: ESSENTIAL OILS AND THEIR CONSTITUENTS AS SKIN PENETRATION ENHANCERS OF ANTILEISHMANIAL DRUGS

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Topical treatments could be useful in non-complicated cutaneous leishmaniasis (CL) cases. For most topically applied pharmaceuticals, penetration through the skin barrier is essential for developing their effects. For example, a transdermal treatment is necessary in cases of CL lesions where parasites live intracellularly on dermal macrophages. Essential oils (EO) and major metabolites derived from plants (MDP) could increase skin penetration of both lipophilic and hydrophilic drugs by interacting with the *stratum corneum*. This work aimed to determine the ability of EO and MDP derived from Colombian plants as permeation enhancers. Eight essential oils (EO1-5. EO8-9, EO19) and 12 major-metabolites derived from Colombian plants (MDP4,7,10,19,21, 22, 24, 31, 33, 35-37) were selected from the BioReto XXI-15:50 scientific program. Permeation studies using full-thickness mice skin were performed in Franz diffusion cells at 32 °C, following the OECD Test Guideline 428. The receptor chamber contained PBS buffer (pH 7.4). Caffeine hydrogels containing 1% w/v of EO or MDP were prepared. At various times over 24 h (1, 2, 4, 6, 24 h), 300 µL of the receptor was withdrawn and replaced with the same amount of fresh PBS buffer. Caffeine was analyzed by a UV-VIS spectrophotometry method at 272 nm. Once the experiments were completed histopathological analysis of the membrane was performed (n=8). We found an increase in the parameters of caffeine permeation in the presence of some EO and MDP. The value produced by the steady-state flux (estimated by linear regression through

the data obtained between one and 24 h) of caffeine gel (control) was 30±19.6 µgcm^{-2}h^{1}. An increase of almost 4-5 times was observed after being combined with EO2 or EO19. The values were 130±47.6 and 150±14.1 µgcm^{2}h^{-1} respectively. In addition, an increase of almost three times in caffeine flux was observed after MDP6, MDP19, and MDP22. The values were 86±21.0; 90±18.4 and 101±21.7 µg cm^{-2} h^{-1} respectively. The potency of caffeine's penetration alone (permeability coefficient Kp) was 800±503.5 cm^{-2} h^{-1}. An increase of almost 2-4 times was observed after being combined with EO2, EO19, MDP6, MDP19 where caffeine Kp values were 2900±1046.1, 1300±469.5, 2100±516.5, and 2200±973.9 cm^{2}h^{-1} respectively. Some changes in the skin epidermis were observed histopathologically at the end of the assay. The EO2, EO19, MDP6, and MDP19 tested were able to improve the permeation of caffeine through the skin layers, suggesting that these compounds may be effective for transdermal delivery of hydrophilic antileishmanial drugs.

Keywords CUTANEOUS LEISHMANIASIS; ESSENTIAL OILS; TRANSDERMAL DRUG DELIVERY; COLOMBIAN PLANTS

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P4-019.1: IN SILICO MOLECULAR DOCKING STUDIES AND ANTILEISHMANIAL ACTIVITY OF FRACTIONS *Malachra alceifolia* AGAINST *Leishmania mexicana* PROTEASES.

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Malachra alceifolia Jacq (family Malvaceae), known "Malva" medicinal plant that is used as a traditional therapy in many regions of América, W. tropical África, and Tropical Asia. Traditionally this plant used in the form of extracts, powder, paste by populations from the northern Colombian for treating fever, stomach, inflammations and parasites. The extraction and chromatographic fractionation leaves extracted by maceration in 98% ethanol (15 L) for 4 days, extracts were chromatographed using open column fractionation on silica gel (16g; column length: 11cm; internal diameter: 2.4cm) using solvent mixtures of increasing polarity as follows: (Hexane/CHCl3, CHCl3, CHCl3/EtOAc, EtOAc, and EtOAc/MeOH). The identification of the components of fractions with major activity biological, an Agilent Gas Chromatograph 7890A series (Agilent Technologies, Inc., Santa Clara, CA, USA) was used. This study evaluated the in silico molecular docking performed by AutoDock Vina inhibitory ability of compounds bioactive fractions present over protein targets *Leishmania mexicana*; leishmanicidal activity axenic amastigotes *Leishmania mexicana pifanoi* (MHOM/VE/60/Ltrod) and cytotoxic activity in the RAW 264.7 murine macrophage cell line. The chemical analysis fractions of *M. alceifolia* leaves revealed bioactive fractions, MAF8C and MAF9-10 with secondary metabolite presence unreported in the literature, alpha-Tocospiro A, alpha-Tocospiro B, gamma-Tocopherol, Alpha-Amyrin, and Methyl commate A in this genus. The docking study provided an insight into the prediction of affinity, activity, binding, and orientation of alpha-amyrin to the target protein Pyruvate kinase of *L. mexicana* 9.9 Kcal/mol. All fractions showed

high for antileishmanial activity against *L. mexicana*, MAF8C and MAF9-10 at 50 µg/mL percentages of survival 4.60% and 16.2%. The MTT assay revealed that bioactive fractions of *M. alceifolia* exerted no significant cytotoxicity in the RAW264.7. The *M. alceifolia* has the potential of active phytoconstituents, which can be used to search for new drugs and molecular targets, Alpha-Amyrin was the compound that showed the best free binding energy shown to have good antileishmanial. Furthermore, further contributions to research, validation, and conservation of traditional knowledge of medicinal plants globally are needed. This research was supported the University of Cartagena, Doctoral program in biomedical sciences from the University of Cartagena and the National Program for Doctoral Formation Minciencias, 727- 2015, Colombia

Keywords: *Malachra alceifolia;* PHYTOCONSTITUENTS; ANTILEISHMANIAL ACTIVITY

5.4. EPIDEMIOLOGY – ECOEPIDEMIOLOGY - MOLECULAR EPIDEMIOLOGY - PREVENTION AND CONTROL

P1-039: SURVEILLANCE FOR VISCERAL LEISHMANIASIS AMONG IMMUNOSUPPRESSED AMERICAN SERVICEMEMBERS PREVIOUSLY DEPLOYED TO IRAQ/AFGHANISTAN

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Transboundary movement of *Leishmania infantum* from Iraq and Afghanistan to the United States, a non-endemic country, by formerly-deployed returning American servicemembers is an emerging issue. Nearly 20% of Iraq-deployed soldiers have been found to have asymptomatic visceral leishmaniasis. Those soldiers who subsequently became immunosuppressed due to medication use, organ transplant, or HIV infection may be at significant risk for activation of this chronic parasitic infection. We investigated this high-risk population for clinical and laboratory evidence of *Leishmania infantum* infection. This surveillance study was conducted at Walter Reed National Military Medical Center (Bethesda, Maryland) and Tripler Army Medical Center (Honolulu, HI) among three American cohorts: 1) tumor necrosis factor-α (TNF-α) inhibitor users, 2) organ transplant recipients, and 3) persons infected with

HIV. All participants had previously deployed to Iraq, Afghanistan, or both. 50 non-deployer controls (58% male; 42% female) from the same populations were also enrolled. Medical history, a risk factor survey, and blood samples were obtained during study visits. DNA extracted from frozen whole blood was assessed with a *Leishmania* REPL repeat region Taqman quantitative PCR. The level of detection was ≥1 parasite/mL. Sera was tested with a *L. infantum* Soluble Leishmania Antigen-based ELISA. Each immunosuppression group had a cutoff value of 2 standard deviation over the mean of the appropriate control group. rK39 (KalazarDetect™, Inbios) immunochromatographic test was performed on positive ELISA and PCR samples. At the time of interim analysis, 39 deployers had enrolled (26 TNF-α users, 8 organ transplant recipients, and 5 HIV-infected persons). All deployers were male. 28 had deployed to Iraq (18 TNF-α, 5 transplant, 5 HIV), 18 deployed to Afghanistan (15 TNF-α, 3 transplant, no HIV) and 7 TNF-α users had deployed to both countries. 3 of 39 (7.7%) participants had low levels of detectable *L. infantum* DNA in their blood (Range: 95–284 copies/mL); all were TNF-α inhibitor users, 2 deployed to Afghanistan. Anti-*L. infantum* SLA IgG responses were observed in another 8 participants (20.5%; one HIV, one organ transplant, 6 TNF-α). Amongst ELISA positives, 3 deployed to Afghanistan; 3 deployed to Iraq; and 2 deployed to both countries. rK39 testing was negative in all PCR or ELISA positive cases. No participants thus far have had symptoms of activated visceral leishmaniasis. Our current findings are consistent with prior studies examining the prevalence of *Leishmania* exposure in previously deployed U.S. servicemembers. There is a similar distribution of country of exposure amongst those with positive PCR and ELISA results, with 4 deployed to Iraq, 5 deployed to Afghanistan, and 2 who have deployed to both locations. All positive PCR results thus far have been found in TNF-α inhibitor users, which is also our largest cohort. In summary, interim analysis of data examining the prevalence of *L. infantum* infection in immunosuppressed U.S. servicemembers with history of deployment to Iraq or Afghanistan suggests that 7.7% have circulating blood parasitemia (PCR positive), and another 20.5% demonstrate a reactive *Leishmania* serology, for an overall positivity rate of 28.2%.

Keywords: VISCERAL LEISHMANIASIS; IMMUNOSUPPRESSION; IRAQ; AFGHANISTAN

P1-041: UNDERSTANDING THE ROLE OF BIOLOGICAL FACTORS INVOLVED IN THE TRANSMISSION CYCLE OF CUTANEOUS LEISHMANIASIS IN AN ENDEMIC COMMUNITY FROM GUATEMALA

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Guatemala is an endemic country for leishmaniasis, with cutaneous leishmaniasis (CL) being the most prevalent clinical form and with few visceral cases reported nationwide. CL incidences in endemic areas have increased from 28.9 – 72.26 per 100,000 inhabitants in the last 10 years. The distribution of the CL cases has also been expanding. Despite the endemicity of the disease in the country, vector incrimination has not been achieved and no reservoir studies have been carried out. More information is available on the *Leishmania* parasite species circulating in human patients. For more than 30 years, the most prevalent parasite species has been *L. braziliensis,* followed by *L. mexicana*. Recently, the species complex *L. guyanensis/panamensis* was also detected. The aim of this study is to improve the knowledge of the biological factors (parasite, vector, and reservoir) involved in the transmission of cutaneous leishmaniasis in an endemic community in Guatemala. Our study is focused on one of the communities that reported the highest incidences of CL in 2021. For the parasites, we will identify the *Leishmania* species causing CL in patients, using tissue smears collected by the Ministry of Health for the parasitological diagnosis of CL. To date, we have collected three tissue smears from patients living in the community. For the vectors, we are currently collecting sand flies in households of suspected or confirmed CL cases with traps placed in intra, peri and extra domiciliary locations. To date, 15 sand flies have been collected, including three females blood fed. Sand fly species will be identified by morphology, confirmed by conventional PCR

targeting the cytochrome c oxidase I (*COI*) gene, and *Leishmania* infection will be assessed. We will use a conventional PCR targeting the heat shock protein, *hsp70,* followed by a restriction fragment length polymorphism analysis (RFLP) to identify the species of the *Leishmania* infecting humans and sand flies, and the *hsp70* amplicons will be sequenced to build a phylogenetic tree. Sand flies infected with the same genetic variant of *Leishmania* found in humans, could be further explored as potential vectors. The gut of engorged sand flies will be analysed by conventional PCR targeting the *COI* gene to assess the source of blood meal as an approach to identifying possible reservoirs. This project is being conducted in close coordination with the National Ministry of Health and the community leaders, and the results will provide basic knowledge to guide further studies on the incrimination of sand fly species as vectors of leishmaniasis and the identification of the CL mammalian reservoir in the region. The identification of a potential vector and reservoir can help not only to guide research efforts, but also the control and prevention measures taken by the community and the Ministry of Health.

Keywords CUTANEOUS LEISHMANIASIS; GUATEMALA; SAND FLY; INCRIMINATION; TRANSMISSION

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P1-042: PROFILE OF TRANSPLANTED PATIENTS AFFECTED BY VISCERAL LEISHMANIASIS IN BRAZIL

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Visceral leishmaniasis (VL) is considered an opportunistic infection in immunocompromised patients, including solid organ transplant (SOT) recipients, in whom there is an increased risk of VL, which can occur after primary infection by the bite of infected female sandflies, via transplanted organs, blood fluids or reactivation of latent infections. Brazil has the largest public kidney transplant program in the world, being the most effective therapy in restoring the quality of life of patients with chronic kidney disease. Emerging infections caused by opportunistic agents are a major challenge, which may compromise the therapeutic success of transplants in the country. A cross-sectional descriptive study was carried out on the profile of transplant patients affected by VL, who used liposomal amphotericin B (LAMB) in Brazil, from 2011 to 2021. VL notifications registered in the LAMB request forms were selected from FormSus and RedCap, with some information regarding transplantation in the following variables: description of the patient's clinical history; other signs and symptoms; LAMB indication criteria; other indication criteria and comorbidities that compromise immunity. The variables were made compatible and duplicate records were excluded, keeping the most recent. A total of 129 cases of VL were identified in SOT recipients, with the highest percentages of kidney (65.9%) and heart (12.4%) transplant recipients. Although the criterion for indicating the use of LAMB was kidney, heart or liver transplantation, in 7.8% it was not possible to identify the type performed. VL was twice as frequent in male than female SOT recipients (85:44), mainly affecting the age groups 50 to 59 years (29.5%) and 20 to

39 years (23.3%). The most frequent signs and symptoms were: pallor (72.9%), fever (69%) and splenomegaly (65.9%). Kidney disease was reported in 66.7% and heart disease in 17.1%. HIV coinfection was confirmed in 7.8% and 22.5% reported other associated diseases such as diabetes mellitus, fungal infection, pneumonia and pulmonary tuberculosis. In 49.6%, there was no information about previous treatment and 45.7% had already been treated with LAMB. The most performed diagnosis for the confirmation of VL was the bone marrow aspirate (47.3%), followed by the rapid test (30.2%). Bone marrow aspirate is one of the most used laboratory methods for the diagnosis of VL in transplant patients, but an approach to be considered is the combination of methods. Even though many cases with classic VL symptoms (fever/splenomegaly), there are reports of patients where VL manifests itself in an atypical way, which may interfere with the early diagnosis of the disease. In this sense, VL should be considered in the differential diagnosis when fever of unknown origin is present in SOT recipients who live in or have traveled to endemic areas. With the growing number of organ transplants being performed and, even though VL is still considered a rare disease in transplant patients, the results point to the importance of carrying out more careful analyzes regarding the presence of this disease, including other sources of information, such as the National Transplant System, medical records, among others.

Keywords TRANSPLANTED; VISCERAL LEISHMANIASIS; IMMUNOCOMPROMISED; SOT

P1-043: A CROSS-SECTIONAL STUDY OF REGIONAL *LEISHMANIA* SPECIES VARIATION IN ECUADOR: CONSEQUENCES FOR CLINICAL PRACTICE AND RESEARCH

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Among eight Cutaneous Leishmaniasis (CL) causing *Leishmania* species in Ecuador, *Leishmania guyanensis* and *Leishmania braziliensis* are dominant. *Leishmania braziliensis* is the only known causative species of mucosal leishmaniasis in Ecuador. Earlier studies on *Leishmania* species in Ecuador focused on the Pacific areas, included few patients from the Amazon region, and did not study patient characteristics. The resulting lack of knowledge impairs region-specific diagnosis and therapy for CL possibly leading to treatment delay, inadequate treatment, patient suffering, and waste of recourses. This study aims to determine the distribution of *Leishmania* species in a subtropical Pacific and Amazon region and to analyze regional differences in CL patient presentation. Confirmed patients were included from January 2019 through June 2021 by private and public primary health care centers and hospitals in the Pacific part of the Pichincha province and the Amazonian Napo, Pastaza, and Morona Santiago provinces. All patients

were subjected to a microscopic smear slide examination of a CL-suspected skin lesion in the participating centers. Patients without *Leishmania* parasite confirmation were excluded from the study and received free medical follow-up. A skin scraping and filter paper imprint sample were taken from the border of the lesion for smear slide microscopy and qPCR. *Leishmania* species was determined by Cytochrome B sequencing in all qPCR-positive samples. Additional patient and geographic variables were collected per patient. All calculations were done in SPSS Statistics version 28, considering P <0,05 as statistically significant. The presence of *Leishmania* parasites was confirmed with PCR and/or microscopy in 245 patients who were included in this study. 154 patients (63%) were infected in the subtropical Pacific region and 91 (37%) in the Amazon. Infecting *Leishmania* species could be determined in 115 (62% of qPCR positives) patients. *Leishmania guyanensis* was the main species (95%) in the subtropical Pacific but more than half of the patients with species determination from the Amazon were either infected by *L. braziliensis* (39%) or *Leishmania lainsoni* (15%). Altitude and humidity at the place of infection did not differ significantly across species. *Leishmania guyanensis* infected samples had significantly (P = 0,02) lower CT values. 73% of the Amazonian CL patients in this study were Amerindian. The mean health-seeking delay for *L. braziliensis*-infected patients was 3 months longer (P <0,01). Lesion type and the number of lesions were not significantly different across species. In the Pacific region, the health-seeking delay was relatively short and *L. guyanensis* was the main causing species leading to a low risk of mucosal leishmaniasis. The majority of CL lesions in the Amazon were caused by *L. braziliensis* or *L. lainsoni*, the health-seeking delay was longer, and the majority of patients were Amerindian. Higher mean CT values in *L. braziliensis* infected samples indicate lower sample *Leishmania* DNA concentrations leading to a possible underestimation of *L. braziliensis* prevalence in this study. We recommend future studies of determinants of health-seeking delay in CL patients and reconsideration of intralesional treatment of CL lesions from the Pacific region.

Keywords LEISHMANIASIS, CUTANEOUS; SPECIES SPECIFICITY; TIME-TO-TREATMENT; GEOGRAPHIC LOCATIONS; ECUADOR

P1-044: MUNICIPAL VULNERABILITY IN THE STATE OF RIO DE JANEIRO/ BRAZIL, FOR TRANSMISSION OF AMERICAN VISCERAL LEISHMANIASIS

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The Brazilian Program for the Surveillance and Control of American Visceral Leishmaniasis (AVL) classifies municipalities with specific control actions recommended for each category. Municipalities without reported human or canine cases in the last three years are considered silent and can be further classified as vulnerable if: 1) share borders with municipalities with transmission, 2) have intense human migration, and/or 3) are part of the same road network. Vulnerable municipalities can be classified as receptive (with records of the vectors *Lutzomyia (Lutzomyia) longipalpis/ L. (L.) cruzi*) or non-receptive (without records of the vector). The State of Rio de Janeiro (RJ) has a small number of human cases of AVL; but it should not be neglected due to the high number of infected dogs, mortality, the vector adaptation, urbanization, and expansion of the disease. Therefore, preventive measures in silent areas are crucial to avoid its spread. This study aimed to identify vulnerable municipalities in RJ and guide future entomological surveys, by mapping the spatial distribution of the disease (human and canine) and its local vector, *L. (L.) longipalpis.* The occurrence of *L. (L.) longipalpis*, human and canine cases of AVL were obtained at the National Information System on Notifiable Diseases, from the Health Department of the State of RJ, from the National Reference Services on Leishmaniasis and from the literature. The data were integrated into a Geographic Information System/QGIS and classified according to the

abovementioned criteria, established by the Brazilian Ministry of Health. In the period of 2011-2022, human AVL occurred in 09 and canine VL in 41 of the 92 municipalities in RJ. In the last three years (2019-2021), 27 municipalities had records of canine VL. Five municipalities had records of human AVL; all classified as sporadic transmission, where Barra Mansa, Rio de Janeiro and Volta Redonda are municipalities with records of canine VL and the presence of the vector *L. (L.) longipalpis*. In the state, 62 (67%) vulnerable municipalities were identified, 09 (8%) of which were receptive, and only one municipality was classified as silent and not vulnerable (Aperibé). Rio de Janeiro has only 17 (18%) municipalities with entomological survey and records of the vector. The transmission of AVL currently occurs in 32% of the RJ and classified as sporadic. Approximately 82% of all the state, and among the vulnerable municipalities, 85% municipalities do not have information on sandflies, which shows a clear need for entomological studies. It is known that notifications about human and canine cases of VL are still precarious, a fact that needs to be reviewed, since they are essential data for surveillance and control actions to be implemented efficiently in the state and in the municipalities. After the detection of the vector in vulnerable municipalities, the recommended control actions are, health education actions, environmental management, and canine investigation, aiming at the early detection of AVL cases. This type of study has as its main perspective to provide support for surveillance campaigns and prevention of AVL transmission, whose model can be applied to different regions of Brazil.

Keywords RIO DE JANEIRO; VISCERAL LEISHMANIASIS; *Lutzomyia longipalpis*, VULNERABILITY; SURVEILLANCE

Financing INCT, CNPq, FAPERJ, Instituto Nacional de Infectologia, Instituto Oswaldo Cruz, FIOCRUZ

P1-045: DEATH BY VISCERAL LEISHMANIASIS: TEMPORAL EVALUATION OF DIAGNOSIS INOPPORTUNITY

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Employees of Ministério da Saúde, SVS

Visceral leishmaniasis (VL) is a serious disease that mainly affects some of the poorest people. Considering that the evaluation of diagnostic indicators are tools that contribute to the control of the disease, the main goal of this study is to describe and evaluate the opportunity of diagnosing cases of human VL, on people who evolved to cure or death, in Brazil. It is an ecological study with an analytical approach. Data were collected in the Sistema de Informação de Agravos de Notificação (Sinan) of VL cases, between the years 2017 to 2019. For the purposes of this study, new and confirmed cases of VL that resulted in cure or death were included. Due to the absence of a variable in the form that indicates the exact date of the diagnosis, a new variable was created, called “diagnosis time” (DT), using the two variables present in the database: “date of initial symptoms” subtracted from the “notification date”, which resulted in a range in days for the DT. For this variable, the average was calculated. The DT was divided between patients who were supposedly diagnosed up to 60 days and patients diagnosed more than 61 days after the initial symptoms. Considering the relevance among the most vulnerable groups for VL, the population of the study was divided into 2 groups: Confirmed cases in children under 5 years old (Group 1) and confirmed cases in individuals over 50 years old (Group 2). To analyze the data pattern, the Epi info program (7.2.4.0) was used and the significant association between the variables was investigated using the Chi-Square test, considering the value for rejection of the null hypothesis of $p \leq 0.05$. Confidence intervals (CI) of 95% in the defined period and the relative risk (RR) were estimated, considering the DT related to the death outcome. In group 1, it was included

2,533 cases, of which 94.1% progressed to cure. On the Other hand, in Group 2, it was analysed 1,321 individuals, in which 76.5% of the cases, the patients evolved to cure. The results of the study revealed an average DT interval of 15 days for group 1 (p=0.0001) and 23 days for group 2 (p=0.02). Therefore, it is inferred that patients of 5 years old diagnosed with VL have 6.9 times (CI 1.52 - 3.42) more risk of evolving to death after 60 days from the date of the diagnosis, compared to those diagnosed before the 60 days. As well as patients over 50 years old have 6.4 times (CI 1.04 – 1.7) more risk for the outcome of death. Although there is a need for a multivariate comparison of the factors that predispose to death, it is assumed that the results obtained by this study reveal that the inopportunity should be a factor to be considered, especially in groups that are more vulnerable to VL.

Keywords DIAGNOSIS; UNOPPORTUNITY; DEATHS

P1-046: DETECTION OF *LEISHMANIA* INFECTION IN BLOOD DONORS AT TWO BLOOD BANK SITES IN NORTHWEST ETHIOPIA

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While *Leishmania* parasites have been found in blood products in visceral leishmaniasis (VL) endemic areas in Latin-America, the Indian subcontinent and Europe, this has never been studied in East-Africa. However, countries such as Ethiopia have found asymptomatic *Leishmania* infection to be common in healthy individuals living in endemic areas. Administering blood products from donors with previous VL infection carries the risk of transmitting *Leishmania* parasites to recipients, which can particularly be a risk to develop VL for immunocompromised individuals. We established the prevalence of asymptomatic *Leishmania* infections and associated socio-demographic factors among blood donors at two blood bank sites in northwest Ethiopia. A cross-sectional study was performed between June and December 2020. Socio-demographic information and a blood sample were collected from voluntary blood donors enrolled consecutively in the study at two blood bank sites: Metema hospital in a VL endemic site, and the University of Gondar hospital in a VL non-endemic area. To test the participants for asymptomatic *Leishmania* infection, blood samples were tested by rK39 rapid diagnostic test (RDT), rK39 ELSIA, direct agglutination test (DAT) and qPCR targeting kinetoplast DNA (kDNA). Asymptomatic infection was defined if one of the tests was positive. A total of 426 voluntary blood donors were included, 215 at Metema Hospital and 211 at the university of Gondar hospital. The median age was 22 years (IQR, 19 – 28 years); 59.4% (n= 253/426) were male. 347 (81.5%) of the 426 donors resided in urban areas. Only one participant had a history of VL and three

with a family history of VL. Asymptomatic infection was detected in 9.9% (95%CI 7.4 – 13.1) of the blood products, with a prevalence of 15.3% (95%CI 11.1 – 20.8) in Metema and 4.3% (95%CI 2.3 – 7.9) in Gondar. The rK39 RDT was positive in 2.6% (n=11/426), rK39 ELISA in 3.5% (n=15/426), DAT in 0.7% (n=3/426) and PCR in 2.6% (n=11/420). There were six patients with two positive tests: one positive on rK39 and PCR and five positive on rK39 RDT and ELISA. The prevalence of asymptomatic infection was higher in Metema (OR 10.34, p= 0.0013) but was not associated with age, sex, a history of VL or living in a rural area. We detected *Leishmania* infection in 9.9% of blood products, including also by PCR. This raises concerns on the risk of transmission of the infection to blood recipients. Screening blood products for *Leishmania* infection should be considered for blood donors living or having lived in VL endemic areas in Ethiopia and other East-African countries

Keywords BLOOD TRANSFUSION, ASYMPTOMATIC LEISHMANIA, BLOOD BANK, SCREENING

P1-048: VISCERAL LEISHMANIASIS IN A MILITARY AREA OF BRAZIL: EPIDEMIOLOGY, SANDFLY FAUNA, RESERVOIRS, AND LEISHMANIA RESEARCH

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Leishmaniasis is a zoonosis of great worldwide prevalence, caused by protozoa of the genus *Leishmania*, which are transmitted by the bite of infected female sandflies. The dog is considered the main reservoir of *Leishmania* in urban environments. The objective of the work was 1) to know the fauna of the sandfly species, and 2) to research *Leishmania* in animals residing in the military area. This is an epidemiological study carried out at the 4th Mechanized Cavalry Brigade of Dourados in the state of Mato Grosso do Sul, Brazil. During a period of one year (January to December/2021), eight CDC traps were installed at dusk and collected at dawn after an average of 13 hours, for three consecutive nights. The sandflies species collected were identified by microscopic analysis. In the animals, a questionnaire with epidemiological and clinical data was applied and peripheral blood was collected for serology (DPP™ rapid test) and Polymerase Chain Reaction (PCR) for *Leishmania* spp. In total, 659 sandflies were included, of which 333 (50.5%) were females. The largest number of insects captured was in January (n=138; 20.9%) and November (n=198; 30.0%). Two points located in a region with preserved vegetation together accounted for 82.7% (n=545) of the specimens collected. So far, 39.6% of the samples of males and 25.2% of females have been identified, with at least seven species being found. Both among males and females, the most prevalent species was *Brumptomyia brumpt* representing respectively 21.7% and 39.3%. The other species found were: *Lutzomyia longipalpis*

(n=10; 4.7%), *Nyssomyia neivai* (n=14; 6.6%), *Nyssomyia whitmani* (n=8; 3.7%), *Bichromomyia flaviscutellata* (n=34; 16.0%), *Psathyromyia aragaoi* (n=33; 15.5%), *Nyssomyia intermedia* (n=12; 5.6%), *Brumptomyia cunhai* (n=15; 7.0%) and *Brumptomyia pintoi* (n=6; 2.8%). Among the animals, all 36 animals were included in the study, being 8 dogs and 28 horses/mares. Most were females (60.6%), without clinical alterations (57.6%). All dogs received three doses of vaccine against leishmaniasis in the 1st year of life. As for the tests, serology was non-reactive in all dogs and PCR was negative in all animals. It is corroborated by several authors that environmental conditions such as sanitation, hygiene, presence of vegetation and natural food source influence the proliferation of vectors and, consequently, the dissemination of *Leishmania* spp. in accidental hosts. The studied area has a rigorous routine of maintenance of hygiene besides the presence of native vegetation containing wild animals such as foxes, ocelots and birds that can serve as a reservoir for the parasite, which are some of the factors that prevent the vector from moving to areas of circulation of dogs, horses and man. Another important factor to be observed is that the analyzed animals have preventive veterinary care and daily contact with their handlers and trainers, facilitating the care. Thus, preventive measures such as environmental preservation, maintenance of the places where the animals are housed and circulate, and veterinary monitoring of the animals, are factors that prevent the proliferation of *Leishmania* spp. in the urban environment.

Keywords *Leishmania*; EPIDEMIOLOGY; FAUNA; PHLEBOTOMINAE

P1-050: PREVALENCE OF ASYMPTOMATIC *LEISHMANIA* INFECTION IN MADRID, SPAIN

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Asymptomatic infection represents approximately 20–60% of *Leishmania* spp. infection in endemic areas. The identification and management of these subjects have become an increasingly important challenge in leishmaniasis control programs. There is no agreed definition of the condition or accurate means by which to detect this asymptomatic population. For this purpose, in this study, we have used molecular, serological, and cellular techniques to determine the prevalence of asymptomatic *Leishmania* infection in Madrid. For this purpose, a cross-sectional study was conducted within the framework of the Madrid region's V Sero-epidemiological Survey. We included 3868 samples from healthy volunteers aged between 1 and 80 years, with no previous history of leishmaniasis and no signs or symptoms of the disease, from 82 public primary healthcare centers. Detection of individuals with asymptomatic infection was performed by 1) the combination of indirect immunofluorescence assay (IFAT) (titer ≥ 1:80) and polymerase chain reaction (k-PCR), and 2) whole blood stimulation assay (WBA) and IL-2 quantification (cut-off ≥ 50.3 pg/ml). Using WBA and IL-2 quantification, we found that age (>41 years) and being male are risk factors for asymptomatic

Leishmania infection. Using this technique, a mean prevalence of 9.0% (320/3548) of asymptomatic infection in the Madrid region was reported. This prevalence was variable, with 5.7% (95%CI 2.8-8.7) in the northern area, 6.6% (95%CI 4.4-8.8) in the central area of Madrid, 9.4% (95%CI 3.5-15.4) in the southern area and 15.0% (95%CI 8.4-20) in the southwest of the Madrid region (where Fuenlabrada is located, a post-outbreak area of leishmaniasis). However, the mean prevalence using IFAT and k-PCR was 0.13% (5/3868) and 0.0% (0/320), respectively; underestimating the cohort of asymptomatic infected subjects in the whole community. We were also able to identify three areas with a high prevalence of asymptomatic *Leishmania* infection in areas outside the leishmaniasis outbreak (Alcala de Henares (21%), Villanueva del Pardillo (15%), and Leganes (21%)). In this epidemiological survey, we determined the prevalence of asymptomatic *Leishmania* infection in the different areas of the Madrid region. Detection of these asymptomatic subjects was most effective using the cellular test (WBA). In addition, these studies have revealed areas with a high prevalence of asymptomatic *Leishmania* infection in which there could be possible foci of leishmaniasis, and therefore it is vital to monitor and control the vectors in these areas.

Keywords ASYMPTOMATIC *Leishmania* INFECTION; WHOLE BLOOD ASSAY; INTERLEUKIN-2; PREVALENCE; SURVEY

Financing This study was funded by the ISCIII (PI18CIII/00028) and by the CSCM

P2-074: NEW OUTBREAK OF CUTANEOUS LEISHMANIASIS TRANSMISSION IN THE MUNICIPALITY OF ANZÁ IN THE DEPARTMENT OF ANTIOQUIA

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Vector-borne diseases are responsible for the greatest economic and social burden in Latin American endemic countries in tropical and subtropical areas. They are considered to be diseases that promote underdevelopment and contribute to the marginalization of populations with little access to public and health services. In Colombia, in recent years, several factors have determined an increase in the transmission of leishmaniasis. The control of these diseases is complex and requires an integral management to make it possible the identification of the population group with the highest risk of infection, the times of the year, hours of the day and place of residence where the highest risk of contact between people and the infected vector is. The research was developed in the hamlets of La Cejita and Higuiná in the municipality of Anzá, which is presenting an unexpected increase in cases of cutaneous leishmaniasis in recent years, so it is necessary to know the dynamics of transmission of the disease. To carry out the study, 18 houses in La Cejita and 28 in Higuiná were geo-referenced and interviewed to characterize the housing conditions using the SW maps application version 2.4.8 and GPS Garmin etrex 10 version 2.0. In addition, the Montenegro test was applied to 47 people in La Cejita with only one positive person and 57 in Higuiná of which 4 were positive. With these results, the prevalence of exposure was calculated for the hamlets under study, being 2.13% for La

Cejita and 7% for Higuiná. Using CDC type light traps in the intra- and peri-domicile, 66 phlebotomine sandflies were captured (37 females and 29 males), distributed in five genera (*Brumptomyia, Evandromyia, Micropigomyia, Lutzomyia* and *Pintomyia*) and six species. From the species collected, *Lu. gomezi* has been identified as a species of medical importance because it has been found infected with *Leishmania* parasites. It should be noted that the specimens captured from La Cejita trail are only of two species, *Pi. nuneztovari* and *Br. mesai*, neither of which are incriminated as vectors. A descriptive analysis was carried out for Montenegro's test and there is no likely transmission of Leishmania in La Cejita, despite finding a positive person in the test with no history of Leishmaniasis, no cases were found, which suggests that the focus of transmission is not active in the hamlet. For Higuiná, the results show that there is a higher risk of infection and, according to the analysis, the transmission is extradomiciliary because positive cases are more associated with work in mining, agriculture and overnight surveillance. It was determined that the residents of the hamlets recognize the disease and its cutaneous manifestations, although its transmission was not associated with phlebotomine sandflies in any case; on the other hand, versions or myths circulate about the infection, which shows that there is a lack of knowledge about the disease cycle. Regarding self-care, apart from the use of appropriate clothing, the use of awnings and repellent, a large percentage of those interviewed do not know how to prevent the disease.

Keywords LEISHMANIASIS; MONTENEGRO, VECTORS

P2-078: A SCOPING REVIEW OF THE LEISHMANIASES IN KENYA

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The leishmaniases are a group of four vector-borne neglected tropical diseases (NTDs) caused by 20 species of protozoan parasites of the genus *Leishmania* and transmitted through a bite of infected female phlebotomine sandflies. Endemic in over 100 countries, the leishmaniases put over 1.6 billion people at risk. The four types of leishmaniasis include visceral leishmaniasis (VL) (also known as Kala-azar), cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and post-kala-azar dermal leishmaniasis (PKDL). In Kenya, the extent of research on VL, CL, MCL, and PKDL remains unclear. This knowledge is instrumental in designing appropriate interventions for diagnosis, treatment and tailoring

strategies for elimination. The present study uses the scoping review methodology to determine the state of leishmaniases research and identify existing gaps in Kenya. Online databases including PubMed, Web of Science, Embase, ClinicalTrials.gov, Cochrane CENTRAL, WHO ICTRP, and the Pan African Clinical Trials Registry (PACTR) were searched to identify all articles published to date discussing VL, CL, MCL, and PKDL in Kenya. Inclusion criteria: articles mentioning leishmaniasis and NTDs in Kenya and any articles mentioning global research on VL, CL, MCL, PKDL and NTDs. A total of 7,486 articles were found. Title and abstract screening was performed to identify studies that specifically discussed the leishmaniases in Kenya, or were likely to. Using this process 479 articles were selected for the full article review. Relevant articles will be included for final analysis and further classified based on type of leishmaniasis (VL, CL, MCL, PKDL), type of analysis (experimental, observational, other), year published, journal published in, relevant themes (pathophysiology, general epidemiology, prevention, diagnostics, treatment, health systems/policy, vectors, co-infections, and general topics) among other classifications. The review has been registered in Open Science Framework.

Keywords LEISHMANIASIS; NEGLECTED TROPICAL DISEASE; KENYA

P2-079: UNDER-REPORTING OF CUTANEOUS LEISHMANIASIS IN AN ANDEAN MUNICIPALITY OF COLOMBIA

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Under-estimation of the incidence of notifiable diseases has two components: under-reporting, which refers to patients who are diagnosed but not reported in the surveillance system; and under-ascertainment, which refers to individuals affected by the disease, but not even diagnosed. Cutaneous leishmaniasis (CL) largely occurs in remote rural areas, with incidence thought to be under-reported by factors of approximately 3 to 5 across the Americas. In Colombia CL is a notifiable disease, and parasitological diagnosis is required for treatment. Here we report initial findings on under-reporting of CL in a municipality in the Andean region of the country. We used three data sources: 1) records of diagnostic laboratories (smear), 2) the Individual Records of Health Service Provision system, known as RIPS for its initials in Spanish, and 3) notified cases in the public health national surveillance system (SIVIGILA). Data were included from 1 January 2017 to 31 January 2019. Non-reported cases were considered those to be in either of sources 1 and 2 but not 3. Unlike sources 2 and 3, source 1 typically does not include ID number or date of birth. For such records we sought matches based on names, allowing for variant spellings using the Oxford Name Compression Algorithm (ONCA) in R software. Most names in Colombia typically consist of two given names (*nombres*) and two family names (*apellidos*). Records in source 1 without an ID number and with at least two of the four names (e.g., one *nombre* and one *apellido*) were considered to be absent from another data source if at most one of the names were matched by ONCA. In sources 2 and 3, records of the same person within 90 days of each other were considered to be from the same case (CL episode). Notified cases in the study period (source 3, SIVIGILA) were 380. Source 2 (RIPS) yielded 183 cases, and a further 125

were found in source 1, for a total of 308. Of these 308, 52 were not found among the 380 records in source 3. So the overall number of cases was 432, of which 12% were not in the SIVIGILA surveillance system. This constitutes our preliminary estimate of under-reporting for CL. Further work will include more refined analysis, e.g., distinguishing multi-record episodes in data source 1, as well as similar analysis of a second municipality, and an estimation of under-ascertainment via data from active community-based case detection using mHealth. Our findings may inform planning of interventions for case management (e.g., purchasing of antileishmanial drugs) and disease control.

Keywords CUTANEOUS LEISHMANIASIS; UNDER-REPORTING; UNDER-ESTIMATION; SURVEILLANCE; COLOMBIA

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P2-082: CHARACTERIZATION OF RELAPSE CASES IN PATIENTS WITH VISCERAL LEISHMANIASIS IN BRAZIL, 2014 TO 2018

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Visceral leishmaniasis (VL) relapse is defined as a resurgence of symptoms within 12 months after clinical cure of the disease. There are studies that show the relapse as a risk factor for death in VL patients, mostly for VL/HIV coinfected patients. The objective of this study is to estimate the VL relapse rate in Brazil from 2014 to 2018 and describe its variation by state, level of endemicity and type of drug used in the treatment of VL. For the descriptive evaluation, secondary data, confirmed cases of VL and HIV in Brazil with the type of entry classified as new or relapsed cases, were obtained from the Notifiable Diseases Information System – SINAN. The following variables were evaluated: demographic (age, sex and race), clinical (presence or not of jaundice, lymphadenopathy, edema, ascites, previous episode of VL, hepatosplenomegaly, associated infectious condition, hemorrhagic phenomenon, pallor, cough or diarrhea, weight loss, pallor, fever, weakness, edema) and co-infection with HIV. In addition, it was evaluated the type of treatment offered, including the medication used. It was calculated point and interval estimates of the number of relapse episodes in the general population with VL and in specific subgroups using the Epi info software (7.2.4.0). During the study period, 5.4% of the reported cases of VL in Brazil were classify as relapse. The states with the highest absolute number of relapses were Maranhão (218 cases), Minas Gerais (203 cases), Ceará (114 cases) and São Paulo (109 cases). The urban perimeter of the municipalities concentrated 80% of relapses and 90% of relapses in the group of LV/HIV co-infected individuals. Almost 40% of relapses reported during the study

period occurred among LV/HIV co-infected individuals, 65% in the age group of 30 and 49 years. In those not coinfected with LV/HIV, the highest number of relapses was concentrated among children between 1 and 4 years old (38%). Males and blacks were the most affected, representing 62 and 59.3% respectively. Regarding to clinical signs, fever, spleen enlargement, weakness and pallor were the most frequent. The cure rate in patients treated with meglumine antimoniate and amphotericin B deoxycholate was similar (69%), while patients treated with liposomal amphotericin B had the highest cure rate, 75%. The factors that are related to relapse in patients with VL have not yet been fully elucidated. However, considering what is described in this summary, it is necessary to identify the variables that are potentially related to relapse so that they serve as a basis for the development of predictive models that can help clinical decisions.

Keywords VISCERAL LEISHMANIASIS; RELAPSE; DEATH

P2-082.1: MONITORING THE EFFECT OF HOUSING CONSTRUCTION ON SANDFLY BITING AND *Leishmania* INFECTION IN NORTHERN ISRAEL: PROSPECTIVE COHORT STUDY

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Over the last 20 years the incidence of cutaneous leishmaniasis (CL) in Israel, caused primarily by Leishmania major and L. tropica has increased markedly. In many cases, these patients' place of residence was adjacent to areas of construction. Entomologic studies have shown that large scale development alters both vector and reservoir animals' habitat, potentially increasing the chances of human infections. Our main objective was to examine whether large-scale construction increases the risk for CL. This hypothesis was tested in a prospective cohort study conducted from 11/2017 to 7/2021 in four rural villages near the city of Karmiel, a known CL focus in Northern Israel. Sites were determined based on a preliminary study, in which villages (Yuvallim and Eshhar) undergoing extensive construction (2014-2015 - Exposed) and villages (Shorashim and Kammon - Unexposed) with limited building were chosen. Participating residents signed an informed consent and after completing a structured questionnaire, smears from suspected sores and blood samples were taken. Participants were sampled again in 2/2019 - 12/2019 (season 2); and in 11/2020 - 7/2021 (season 3). Leishmania morbidity was tested by skin smears from suspected wounds were assessed for Leishmania by ITS1-PCR.

Exposure to parasites was examined using antigen-specific T-cell proliferation (TCP) and sand fly biting activity measured by ELISA using sandfly salivary antigens (SSA). Environmental data were collected via self-reports, environmental unit of local authority, and personal observations. Participants with prior or active CL were excluded from the study. Differences between groups were tested using Chi-square test or Mann–Whitney U test, depending on the variable's distribution. Analysis of TCP and antibodies to SSA was performed with Friedman's test due to their non-normal distribution and pairwise comparisons analyzed using the Wilcoxon signed-rank Test with a Bonferroni correction. Overall, 508 subjects fulfilled the inclusion criteria, 363 (71.5%) in the exposed group and 145 (28.5%) in the unexposed group. Compliance rate was 90.9% and 77.4% in seasons 2 and 3, respectively. Over 20 complaints on rock hyrax populations near houses were reported (21 in exposed vs 4 in unexposed villages). Though no new CL cases were detected, the mean TCP of the total sample increased over time ($\chi2=9.568$, $p=0.008$). Likewise, the mean TCP of the exposed group increased with time ($\chi2=10.052$, $p=0.007$); and significant differences of the median TCP were found between the 1st and 3rd seasons (0.90 and 1.10 respectively, $p= 0.006$). No consistent change in mean TCP over time was detected for the unexposed group. Even though no new cases of CL were detected in the exposed villages, the significant increase in parasite infection and numerous complaints regarding large reservoir host populations supports the conclusion that risk of CL goes up following extensive construction and may stay high for several years in endemic region.

Keywords CUTANEOUS LEISHMANIASIS; *Leishmania tropica*; URBAN DEVELOPMENT; T-CELL PROLIFERATION

P3-047: DESCRIPTION OF AN OUTBREAK OF CUTANEOUS LEISHMANIASIS TRANSMISSION IN THE BARCINO HAMLET IN THE MUNICIPALITY OF CAMPAMENTO IN THE DEPARTMENT OF ANTIOQUIA

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Vector-borne diseases are responsible for the greatest economic and social burden in Latin American endemic countries in tropical and subtropical areas. They are considered to be diseases that promote underdevelopment and contribute to the marginalization of populations with little access to public and health services. In Colombia, in recent years, several factors have determined a combination of ecological conditions for an increase in the transmission of leishmaniasis. The control of these diseases is complex and requires an integral management that includes the active search, diagnostic and early treatment of cases; determination of the behavior of the vectors and the establishment of the epidemiological risk of infection in the study area. This information makes it possible the identification of the population group with the highest risk of infection, the times of the year, hours of the day and place of residence where the highest risk of contact between people and the infected vector is. The investigation was developed in a rural area of the municipality of Campamento, in the village of El Barcino. This hamlet was selected because a considerable amount of cases has been reported in recent years according to epidemiological surveillance reports from SIVIGILA. Twenty-one households were geo-referenced and interviewed to

characterize the housing conditions using the SW maps application version 2.4.8 and GPS Garmin etrex 10 version 2.0. In these, the Montenegro test was applied to 59 people (30 women and 29 men), whereof 12 were positive. With these results, the prevalence of exposure was calculated to be 20%. Phlebotomine sandflies were captured with CDC-type light traps in the intra and peri-domicile; they were turned on from 18:00 hours until 06:00 the following day. A total of 28 phlebotomine sandflies (21 ♀ and 7 ♂) were captured, distributed in 4 genera (*Lutzomyia, Nyssomyia, Trichopygomyia* and *Warileya), Lu. hartmanni* and *Ny. trapidoi* have been incriminated as species of medical importance as it has been found infected with *Leishmania* parasites. Using the SAS system version 14.2 package for statistical procedures, a descriptive analysis was performed using Montenegro's test results, showing that the occupation in agriculture (58.33%) of the positives, being a male (66.67%) and the presence of animals such as dogs, cats and pigs in the peridomicile are associated with previous contact or exposure to leishmaniasis. No recent cases of the disease were found, which suggests that the outbreak of transmission is not active in the village, and also no children under 5 years of age were found to be positive for Montenegro, which highlights that transmission occurs outside the home. Although the residents recognize the disease and its cutaneous manifestations, they do not associate the transmission of the disease to the vector, which indicates that there is no knowledge of the disease cycle or its forms of transmission. Concerning self-care, apart from the use of appropriate clothing for work, people do not know how to prevent Leishmaniasis or associate the prevention with other methods to avoid the transmission of different diseases.

Keywords LEISHMANIASIS; MONTENEGRO; VECTORS; FOCI

P3-051: EPIDEMIOLOGICAL PROFILE OF CUTANEOUS LEISHMANIASIS IN ENDEMIC MUNICIPALITIES IN THE INTERIOR OF BAHIA, BRAZIL

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Cutaneous Leishmaniasis (CL) remains an international health concern given its intrinsic relationship with socioeconomic and environmental inequalities. To verify the evolution of the time span of the incidence of reported cases of CL, in Presidente Tancredo Neves, Teolândia and Valença. Backward, individual cross-sectional study, based on cases of leishmaniasis reported in SINAN, from 2011 to 2020. Annual incidence rates (per 10,000) and percentages were calculated according to the different strata of each variable of interest. The incidence of reported cases of leishmaniasis among the municipalities showed great variation, with an average of 42.8/10,000 in Presidente Tancredo Neves, 99.07/10,000 in Teolândia and 16.42/10,000 in Valença. The most affected were male and in the age groups of 15 - 29 and 30 - 59 years in the three municipalities, and an average of 50.0% of the cases were work-related. The occurrence in childhood (0 to 9 years) stands out, around 15.0% in the municipalities. The diagnoses were essentially based on clinical criteria. Almost 100.0% of the patients used antimonials and reported low treatment abandonment rates in Teolândia (0.2%), Valença (0.1%), and Presidente Tancredo Neves (12.3%). The magnitude of leishmaniasis cases in these municipalities calls for greater investments in health and environmental policies and prevention programs, as well as the strengthening of the comprehensive care network regarding the identification, notification, and treatment of cases.

Keywords CUTANEOUS LEISHMANIASIS; NOTIFICATION; INFORMATION SYSTEMS

P3-052: DISTRIBUTION OF *LUTZOMYIA* SPP: A TOOL FOR THE EPIDEMIOLOGY OF VISCERAL LEISHMANIASIS IN URUGUAY, 2021

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Visceral leishmaniasis is a zoonotic parasitic disease that has a great impact on public health worldwide and with a high morbidity and mortality (95% of cases without treatment). The etiological agent involved in the Southern Cone of America is *Leishmania infantum*, being *Lutzomyia longipalpis* (Diptera, Psychodidae) its main vector. Since the 1920s, the presence of two species of the genus Lutzomyia in our country has been known, with specimens of *L. gaminarai* having been found in the departments of Tacuarembó and Salto and *Lutzomyia (Evandromyia) cortelezzi* in Montevideo. In the 21st century, a new species was added. In 2010, the presence of specimens of *L. longipalpis* was reported in the north of the country, in the departments of Salto (city of Salto) and Artigas (city of Bella Unión). Based on these initial findings, an annual search for phlebotominae is carried out in the populated areas near both cities. The geographical distribution of the vector has increased over the years from these first infested cities, always maintaining a spatial continuity. In 2019, the first specimens of *L. longipalpis* were reported in the city of Rivera, capital of the department of the same name. The city of Paysandú has been monitored, given its proximity to the Uruguay River, its close relationship with the Department of Salto and the presence of infected dogs there. However, no phlebotominae have been captured in that locality, to date. The discovery of several specimens of *Lutzomyia gaminarai* in the city of Paso de los Toros,

Department of Tacuarembó and in the city of Artigas (a species that is not implicated in the transmission of *Leishmania infantum*) deserves a special mention. Since a gradual increase in the distribution of *Lutzomyia* spp. in our country has been noted, its presence should be monitored periodically to know its distribution and expansion, its ecology and the epidemiological risk that its presence represents in the different localities. This allows health authorities to take important measures in the management of canine and human visceral leishmaniasis.

Keywords: PHLEBOTOMINAE; URUGUAY; LEISHMANIASIS.

P3-053: ECOPIDEMIOLOGICAL ASPECTS IN A FOCUS OF TRANSMISSION OF CUTANEOUS LEISHMANIASIS FROM VALLEDUPAR, DEPARTMENT OF CESAR

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Leishmaniasis is a neglected tropical disease associated with poverty, and disproportionately affects the poorest communities worldwide. In the capitol of Cesar department (Valledupar), there has been an increase in cases in endemic areas and the appearance of new areas with transmission of visceral leishmaniasis. The goal of the study was to carry out ecoepidemiological surveillance of leishmaniasis in the village of Murillo, peri-urban and rural area from Valledupar, through the implementation of GIS tools and a Leishmaniasis classical focus study. A descriptive study was carried out using a mobile application developed for this purpose in order to collect field information, besides information about the dwellings and the environment, sociodemographic aspects, and knowledge, attitudes and practices of the population about Leishmaniasis. Sandflies were collected using CDC light traps in intra, peri and extradomicile environments of the houses. For the diagnosis of canine visceral leishmaniasis (CVL), rapid Kalazar detect tests were applied, and search for active cases of cutaneous leishmaniasis was carried out, samples were taken from dogs and patients was also tested by PCR. Initial screening for *Leishmania* was carried out by amplifying the ITS1 ribosomal region using primers LITSR and L5.8S, species identification is currently in progress by sequencing of this genetic region and a modified protocol of PCR-RFLP of Leishmania hsp70 gene. The houses from this village are built with wooden walls, zinc roof, and a soil floor. They do not have energy powergrid, aqueduct and sewage service, with open air excreta, burning and garbage disposal. Forest patches around the houses are very common and people are self-identified as farmers

(59%), housewives (38%) and students (3%). From the 57 dog samples, 6 were positive to *Leishmania* by PCR (10,5%) and only one of them tested positive to serological rapid tests. Sandflies associated to CL cases were identified as *Lutzomyia evansi, Lu. shannoni, Lutzomyia gomezi, Lu. dubitans, Lu. rangeliana, Lu. punctigeniculata* and *Lu. trinidadensis. Leishmania* detection in sandflies and *Leishmania* species identification is currently an ongoing work. The presence of visceral leishmaniasis proven vector *Lu. evansi*, and other species highly anthropophilic species such as *Lu. gomezi* and *Lu. shannoni*, and the infection of dogs by *Leishmania* are recorded into the GIS systems and share with the connected users from local health authorities on real time. There is a lack of knowledge about the risk factors, protective behaviors and housing conditions in many Leishmaniasis endemic areas from Colombia due to a high rurality and harsh conditions. These factors usually can limit the success of control measures to be apply because the lack of information available of each area where cases are reported, because of that we think that the development of the GIS and coupled to classical studies may facilitate the implementation of a cooperative prevention strategies with community participation.

Keywords GEOGRAPHIC INFORMATION SYSTEM; VALLEDUPAR; CANINE LEISHMANIASIS; SANDFLIES.

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P3-054: EPIDEMIOLOGICAL PROFILE AND PREVALENCE OF *LEISHMANIA* SPP. IN AN ENDEMIC AREA OF TEGUMENTARY LEISHMANIASIS IN RONDÔNIA, NORTH BRAZIL

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In the American Continent, Brazil concentrates the largest number of cutaneous and mucosal human leishmaniasis (CL/ML) cases and most of the cases are concentrated in the North region of the Country, which presents a wide diversity of circulating vectors and *Leishmania* species. Several *Leishmania* species are associated with human CL/ML leishmaniasis in the American endemic regions, belonging to both subgenera, *L.* (*Leishmania*) and *L.* (*Viannia*). In Brazil only one *L.* (*Leishmania*) species is linked to human CL/ML – *L. amazonensis*, and only two *L.* (*Viannia*) species are not circulating in the Country. In Rondônia state, about 1,000 new cases are registered per year, with autochthony in all municipalities. In this study we analyzed clinical and demographic data of 672 patients diagnosed with CL/ML attended in a reference hospital in Porto Velho, Rondônia, from 2012 to 2022. A total of 586 (87.20%) patients presented CL (localized, disseminated, or diffuse), but 11.76% (n=79) were associated to ML. A small portion of patients, 1.04% (N=7), had concomitant cutaneous and mucosal lesions. Demographic data indicates that 589 (87.65%) patients were male, with prevalence in the 18-40 age group (N=558; 83.03%). However, among female patients (n= 83; 12.35%), highest prevalence was observed in the 41-60 age group (N=38; 46.29%). The identification of *Leishmania* species was performed by *hsp*70-PCR-RFLP patterns or sequence analysis of a 234bp hsp70 fragment. The results indicate a predominance of *L. braziliensis* (n= 557; 82.93%) followed by *L. guyanensis* (n= 52; 7.72%). Another five species were identified: *L. amazonensis* (N=8; 1.19%), *L.*

lindenbergi (N=8; 1.19%), *L. lainsoni* (N=7; 1.04%), *L. shawi* (N=7; 1.04%), *L. naiffi* (N=1; 0.14%). For 32 samples (4.75%), *hsp*70-RFLP profiles were incompatible with known species and, due to DNA loss or degradation, it was not possible to perform identification by sequencing. Our results reenforce the prevalence of *L. braziliensis* followed by *L. guyanensis* in the Northwest of Brazil, but described also the occurrence of human disease linked to other five species. *Leishmania lindenbergi* was recently described in this region, but the protocol of PCR-RFLP-hsp70 employed here does not allow to distinguish between *L. lindenbergi* and *L. guyanensis*. Thus, all samples presenting profiles compatible with these two species were sequenced and it was interesting to observed that 8 patients were infected by *L. lindenbergi*, all of them presenting CL. Although more studies are still needed to link *Leishmania* species to the response to available therapeutic schemes and to the clinical course of the disease, it is already a recommendation by PAHO to perform *Leishmania* species identification to define the best therapeutic approach and follow up conduction. In regions like the one studied here the diversity of parasites observed make this an essential step for the best prognosis of the disease.

Keywords TEGUMENTARY LEISHMANIASIS; *Leishmania* SPECIES IDENTIFICATION; *HSP*70-PCR; RFLP; SEQUENCING

Financing Instituto Nacional de Epidemiologia na Amazônia Ocidental (INCT-EpiAmO)

P3-056: ECO-EPIDEMIOLOGICAL FINDINGS FROM AN EMERGENT FOCUS OF CUTANEOUS LEISHMANIASIS IN YUCATAN, MEXICO

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Localized cutaneous leishmaniasis (LCL) is a zoonotic disease endemic in Mexico. In 2015, we reported the emergence of LCL autochthonous cases in the eastern part of the Yucatan state. It is well known that the establishment of the *Leishmania* transmission cycle depends on the relative abundance of vectors and reservoir species in a particular region. Thus, in this work, we conducted an eco-epidemiological study to determine the elements that favor the establishment of the emergent focus in the municipality of Tinum, Yucatan, Mexico. We implemented a strategy for the epidemiological survey of both clinic and asymptomatic cases of *Leishmania* in collaboration with the Health Services of the Yucatan State. Then, we conducted fieldwork to identify the species of mammals and vectors infected by *Leishmania* parasites. From 2018 to 2021, five cases of LCL were detected. Patients were predominantly males (4/5, 80%) between 30 and 69 years of age working in the sylvatic areas surrounding the locality. A prevalence of 27% (20/74) of asymptomatic infection was found mainly in men who report spending between one and twelve hours working in the forest. The 7SLRNA gen of *Leishmania* was amplified in five of fifteen (33%) asymptomatic individuals. Sixty-seven rodents were captured belonging to five species. *Ototylomys phyllotis* (31/67, 46.2%) and *Heteromys gaumeri* (27/67, 40.2%) were the most abundant species. Of these, twenty specimens were tested by PCR to

identify *Leishmania* infection. Parasite DNA was found in 55% (11/20) of rodents; the proportion by rodent species was 63.6% (7/11) for *H. gaumeri* and 44.4% (4/9) for *O. phyllotis.* Parasites were isolated in culture from 19 rodents (six *H. gaumeri* and 13 *O. phyllotis*). From November 2020 to March 2021, 7,738 sand flies were captured, including 10 species distributed in 6 genera. *Lutzomyia longipalpis, Lu. cruciata,* and *Psatiromyia cratifer* were the most abundant species caught in the Shannon trap and *Leishmania* infection was detected in 48.3% (97/201) of *Lu. cruciata* specimens. *Leishmania mexicana* was identified as the infecting species in humans, rodents and sand flies. Although a low number of LCL cases was detected, our results confirm the establishment of *Leishmania mexicana* transmission in the municipality of Tinum, Yucatan. Our results suggest that *O. phyllotis* and *H. gaumeri* are participating in the transmission cycle of *L. mexicana* in this emergent focus. *Lutzomyia cruciata* could be one of the most important vectors in the area. These findings are useful to implement educational programs for the prevention of LCL in the Yucatan state.

Keywords CUTANEOUS LEISHMANIASIS; *Leishmania mexicana*; WILD RODENTS; SAND FLIES; YUCATAN

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P3-057: DURATION AND DETERMINANTS OF THE LATENCY TIME OF CHAGAS DISEASE IN A COLOMBIAN COHORT

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There is consensus among researchers who have assumed that between 20-30% of patients with Chagas disease (CD) in the indeterminate chronic phase (ICF) will develop cardiopathy, after a period of 10 to 30 years after infection with the parasite, however, this estimate has not been supported by scientific evidence. The objective of this study was to determine the time elapsed from having lived in an endemic area until the development of heart disease in a study of Colombian patients serologically diagnosed with CD. Analytical retrospective cohort study. The information was obtained from the medical records of patients who consulted the National Institute of Health with a serological diagnosis of ICF. The inclusion criteria will be i) being over 18 years of age ii) having been diagnosed with CD by means of two positive serological tests (IFI, HAI or ELISA) iii) having a complete medical history. Patients who were not followed up for at least one year or without a paired electrocardiogram (ECG) during follow-up were excluded. Patients with ICF were followed up annually until December 2020. The endpoint studied was progression to the cardiac form defined by the appearance of electrocardiographic changes typical of CD. Univariate and multivariate survival analysis were performed to identify predictors of CD progression and were expressed as Time ratios (TR) with 95% confidence intervals (95% CI). A total of 578 patients were included, 361 women (62.5%), age range 18 to 88 years (mean 49.8 ± 13.6). In relation to the time lived in an endemic area, 79 (13.7%) lived <5 years; 112 (19.4%) between 5 and <15 years; 200 (34.6%) >15 and <30 years; and 187 (32.4%) >30 years. Three hundred nine patients progressed to heart disease (53.5%). The most common ECG abnormalities were: sinus bradycardia, right bundle branch block, and ventricular extrasystoles. The median time from having lived in an endemic area to progression to heart disease was significantly

shorter in the group of patients who lived <5 years (21.6 years), followed by those who lived between 5-<15 years and >15-<30 years, with median times of 25.7 years and 31.2 years, respectively. Being much longer for those who lived more than 30 years (39.4 years). Possible risk factors for heart disease progression were: male sex (TR 0.82; 95% CI: 0.74-0.91); presence of comorbidities (TR: 0.79; 95% CI: 0.69-0.90); presence of DTU I (TcISYL) (TR: 0.81; 95% CI: 0.71-0.92); having lived between 5-<15 years in an endemic area (TR 1.26; 95% CI: 1.06-1.51); >15-<30 years (TR: 1.20; 95% CI: 1.02-1.43); and >30 years (HR: 1.20; 95% CI: 1.01-1.43) compared to having lived <5 years. In contrast, having received treatment is a factor that increases the time until the progression of the disease (TR: 1.48; 95% CI: 1.34-1.63). The median time to progression to cardiopathy varies according to the time lived in an endemic area, this time being longer in the groups that received treatment. Possible risk and protective factors for the development of cardiopathy are presented.

Keywords CHAGASIC HEART DISEASE; *Trypanosoma cruzi*; CHAGAS DISEASE; COLOMBIA

P4-022: IMPACT OF INTENSIFIED CONTROL ON VISCERAL LEISHMANIASIS IN A HIGHLY-ENDEMIC DISTRICT OF BIHAR, INDIA: AN INTERRUPTED TIME SERIES ANALYSIS

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Visceral leishmaniasis (VL) is declining in India, and the World Health Organization's (WHO) 2020 'elimination as a public health problem' target has nearly been achieved. Intensified combined interventions might help to reach elimination, but their impact has not been assessed. WHO's Neglected Tropical Diseases 2021–2030 roadmap provides an opportunity to revisit VL control strategies. We estimated the combined effect of a district-wide pilot of intensified interventions in the highly-endemic Vaishali district, where cases fell from 3,598 in 2012–2014 to 762 in 2015–2017. The intensified control approach comprised indoor residual spraying with improved supervision; VL-specific training for accredited social health activists to reduce onset-to-diagnosis time; & increased Information Education & Communication activities in the community. We compared the rate of incidence decrease in Vaishali to other districts in Bihar state via an interrupted time series analysis with a spatiotemporal model informed by previous VL epidemiological estimates. Changes in Vaishali's rank among Bihar's endemic districts in terms of monthly incidence showed a change pre-pilot (3rdhighest out of 33 reporting districts) vs. during the pilot (9th)

(p<1e-10). Counterfactual model simulations suggest an estimated median of 354 cases (IQR 234–479) were averted by the Vaishali pilot between January 2015 & December 2017. Our robust analyses show that observed VL case counts fell more quickly in Vaishali district than others (with strong statistical evidence). Thus, corroborating previous crude analyses previously presented by Kumar et al. at WorldLeish6. Additionally, we estimate a 'cases averted' outcome indicator. Strengthening control strategies may have precipitated a substantial change in VL incidence in Vaishali and suggests this approach should be piloted in other highly-endemic districts.

Keywords SPATIOTEMPORAL; ELIMINATION; INTEGRATED CONTROL; DISTRIBUTED-LAG; REGRESSION DISCONTINUITY

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P4-024:STRATIFICATION AND FOCUSING TO GUIDE THE PLAN TO ELIMINATE VISCERAL LEISHMANIASIS AS A PUBLIC HEALTH PROBLEM IN NEIVA. COLOMBIA, 2021

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Neiva is the capital of the department of Huila, Colombia, located in an area of tropical dry forest on the Magdalena River valley and inhabited by 370,318 people. Since 2009, persistence in the transmission of visceral Leishmaniasis has been identified in both rural and peri-urban areas, accumulating 27 cases (1 deceased), all under 5 years of age and identifying *Leishmania infantum* and *Lutzomyia longipalpis*, without a history of characterization, stratification and targeting of areas and risk. The objective of this research is to carry out risk stratification and targeting to guide the elimination plan for visceral leishmaniasis as a public health problem in the municipality. The methodology established in the procedures manual of the Pan American Health Organization (OPS) was developed, stratifying 100% of areas (n=385, -264 neighborhoods and 121 settlements-), which was adjusted in: **I.** Quadrants of 200 meters were created, which would allow establishing the points to intervene according to landscape criteria, **II.** Houses separated by a minimum of 50 meters that met the ideal eco-epidemiological conditions for the presence and abundance of the vector were selected by means of capture with CDC traps and subsequent identification of species according to the Young and Duncan taxonomic keys. **III.** Parasitic circulation was evaluated with the serological diagnosis of the RK39 Kalazar test in 2,205 canines, thus obtaining the simultaneous survey of vectors and canine visceral leishmaniasis. Stratum 1 was established as non-vulnerable, non-receptive and non-transmission areas; stratum 2 the vulnerable, non-receptive and non-transmission areas; stratum 3 the vulnerable, receptive and non-transmission areas; stratum 4 the vulnerable,

receptive and transmission areas. The canine prevalence was 5% (n=111), being higher than 8% in commune 9 (Bethel, Alto Mirador and Bajo Mirador settlements), higher than 3% in commune 10 (Machines settlement and Camelias neighborhood) and higher than 13% in commune 2 (Community Farms settlement); Simultaneously, the registration and taxonomic identification of the *Lutzomyia longipalpis* vector was achieved. It was established that 42.6%, 35.8%, 6.4% and 11.4% of areas of the municipality are located in risk strata 1, 2, 3 and 4 respectively. It is concluded that there is a high risk of urbanization of visceral leishmaniasis as a result of construction in peri-urban areas, which are located in the shape of a horseshoe around the urban area of the municipality. The present study identified the areas in which interventions should be initiated and surveillance should be sustained in order to eliminate the disease as a public health problem and contribute to establishing the route for its escalation.

Keywords LEISHMANIASIS; PUBLIC HEALTH; STRATIFICATION

P4-026: EVALUATION OF MOSQUITO NETS IMPREGNATED WITH LONG-LASTING INSECTICIDE FOR *LUTZOMYIA LONGIPALPIS*, VECTOR OF *Leishmania infantum* IN SOUTH AMERICA

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Mosquito nets impregnated with long-lasting insecticide (LLINs) are used for individual protection and prevention of diseases transmitted by vector, especially malaria. Considering the overlap areas of malaria and leishmaniasis (visceral and tegumentary), the use of LLINs may be an interesting tool of control for both diseases. The objective of this work was to evaluate, in a wind tunnel, the effect of LLIN s as a barrier for *Lu. longipalpis*, the main vector of visceral leishmaniasis in South America. The insects were located inside the wind tunnel, 60 cm from a volatile stimulus and with one mesh in the middle. Three groups were performed: an "intervention A" group, with the mesh impregnated with alpha-cypermethrin (Interceptor®); an "intervention B" group with the mesh impregnated with alphacypermethrin and chlorfenapyr (Interceptor G2®) and a "control" group with the mesh without insecticide. The insects were released in groups of 10, five males and five females, with 12 replications per treatment. The number of insects that passed through the screen was evaluated, up to 60 min from the beginning of the test. In total, 360 insects were used in 12 replicates for each screen (120/screen) and the percentage of insects that passed through each screen was: 58% on the control screen; 79% on the Interceptor screen and 68% on the Interceptor G2 screen. The results indicated that the insects passed through the screens, regardless of whether or not they are impregnated with insecticides. Besides, the number of insects which passed was always greater than those that did not passed in any treatment. Regarding the time of passage of insects, it was observed

that in the two screens with insecticide the insects passed more quickly, with a peak in the first 5 min. No difference was observed in the curve of mortality between the insects that passed through the three screens; however, for the insects that did not passed through the screens, 50% of the population died between 4 and 5 days in the Interceptor, 6 to 7 days in the Interceptor G2 and 9 days in the control. It is concluded that there is no laboratory evidence that points to the barrier effect of the use of mosquito nets impregnated with the mentioned insecticides or the control of *Lutzomyia longipalpis*. However, additional tests are necessary to evaluate the insecticidal effect on the population exposed to the LLINs and the minimum time to cause this mortality.

Keywords *Lutzomyia longipalpis;* CONTROL; MOSQUITO NETS; INTERCEPTOR

P4-027: DIAGNOSTIC AND TREATMENT DELAYS IN VISCERAL LEISHMANIASIS: A MAJOR CHALLENGE FOR LEISHMANIA CONTROL

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In India, visceral leishmaniasis (VL) elimination program has been operating since 2005 and is predominantly based on the optimal functioning of the public sector, and assume that patients will attend public health facilities, attracted by the provision of free drugs. Importantly, a long duration between onset of symptoms and initiation of treatment has been identified as one of the main drivers of the epidemic in mathematical models. Development of a strategies to reduce the delays (patients and system) remains a critical priority to decrease clinical disease and mortality and facilitate eradication. Therefore, to achieve and sustain VL elimination, patients' health seeking behavior and the health system's response will become decisive factors. The present study aimed to investigate the determinants responsible for delays in diagnosis and treatment. We retrospectively calculated the delay between the onset of symptoms and the first contact with a health provider, delays from that first contact to diagnosis and treatment of 4457 VL patients (Age: 23.54 ± 17.69) during last 10 years at KAMRC hospital, Muzaffarpur, Bihar, India. Median delay in first contact, diagnosis and treatment were 5, 42 and 2 days respectively. The delay in diagnosis and treatment correlates with increases in age of patients. Findings of this study suggest that active VL case detection surveillance should be adopted as priority in VL control program.

Keywords VISCERAL LEISHMANIASIS; PATIENTS DELAY; ELIMINATION, HEALTH SEEKING BEHAVIOR

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P4-028: EFFECTS OF MOONLIGHT ON SAND FLY BIOLOGY ACTIVITY IN RIO DE JANEIRO, BRAZIL.

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Sandflies are dipteran insects of the Psychodidae family, vectors of the protozoan *Leishmania* spp. etiological agent of leishmaniasis. The influence of the moon on the biology of these insects is a factor evaluated by several authors who point out the influence of the light incidence generated by the lunar phases interferes in the frequency of these dipterans. However, in studies carried out in open areas, they associate the density of sandflies with the competitiveness of moonlight with the light source of the trap, as well as with the attraction of males by female pheromones. In order to deepen the evaluation of the moon's influence on the biology of these insects, we evaluated the frequency and activity of sandfly species in the Três Picos State Park, in Cachoeiras de Macacu, Rio de Janeiro – RJ, Brazil according to the lunar phases. Monthly captures were performed using CDC light traps, HP model, between 6pm and 6 am of the following day, during two consecutive nights at different monitoring stations. The identification of sandfly species was carried out based on the analysis of morphological characters using the dichotomous key of Galati (2019). The highest dominance of sandflies captured in the park was observed during the periods of crescent moon (39%) and full moon (29%). The species *Bichromomyia flaviscutellata*, incriminated as a vector of *Leishmania (L.) amazonensis*, showed the density (82 specimens) in the new moon period. The results suggest that the lunar phases can influence the capture of sandflies, however, they do not corroborate the hypothesis of other studies where the new moon provides a higher frequency of sandflies in captures

with light traps. However, it can be observed that different species have different activities in the periods of new and full moon and these data are correlated with the activity of mammals that prefer food on sandflies, where small mammals present greater activity in the period of new moon, in agreement with the food preference of *Bi flaviscutellata*. On the other hand, mammals that show greater activity during the full moon period are associated with the food preference of species with greater density in this period, such as *Psydochopygus hirsutus hirsutus*. In addition to these factors, the nights of the full moon provide prolonged light within the forest, causing a luminosity similar to evening twilight, a period of greater activity for sandflies, thus expanding the activity time and consequently greater density. Thus, it is suggested that the luminosity of the moon can influence the activity of sandflies considering different factors and in a different way for each species. This evidence suggests hours of higher risk for human exposure to the transmission of leishmaniasis.

Keywords MOON FASES; PHLEBOTOMINAE; BIOLOGY; TRANSMISSION OF LEISHMANIASIS

Financing CAPES; INSTITUTO OSWALDO CRUZ/ FIOCRUZ

P4-030: EFFICACY OF FACTORY-TREATED AND DIP-IT-YOURSELF LONG LASTING INSECTICIDE-TREATED NETS AGAINST LEISHMANIASIS VECTORS IN THE SUB-ANDEAN REGION, COLOMBIA

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Long lasting insecticide-treated nets (LLINs) may be effective for vector control of cutaneous leishmaniasis (CL). Their efficacy, however, has not been sufficiently evaluated. The purpose of this study was to evaluate the large-scale efficacy of LLINs on *Lutzomyia longiflocosa* entomological parameters up to two years post-intervention in the sub-Andean region of Colombia. A matched-triplet cluster-randomised study of 21 rural settlements, matched by pre-intervention *L. longiflocosa* indoor density was used to compare three interventions: dip it yourself (DIY) lambda-cyhalothrin LLIN, deltamethrin LLIN, and untreated nets (control). Sand fly indoor density and feeding success were recorded using CDC light trap collections at 1, 6, 12 and 24 months post-intervention. It was found that both LLINs reduced significantly (74-76%) the indoor density and the proportion of fully engorged sand flies up to two years post-intervention without differences between them. Residual lethal effects of both LLINs and the use of all nets remained high throughout the two-year evaluation period. It was concluded that both LLINs demonstrated high efficacy against *L. longiflocosa* indoors. Therefore, the deployment of these LLINs could have a significant impact on the reduction of CL transmission in the sub-Andean region. The DIY lambdacyhalothrin kit may be used to convert untreated nets to LLINs increasing coverage

Keywords SAND FLIES; PREVENTION AND CONTROL; BED NETS; LAMBDA-CYHALOTHRIN; DELTAMETHRIN

Financing Instituto Nacional de Salud, Universidad de La Salle and Minciencias, Colombia

5.5. IMMUNOLOGY - CELL BIOLOGY – PATHOGENESIS - VACCINES

P1-052: *IN VITRO* AND *IN VIVO* BIOLOGICAL BEHAVIOR OF *Leishmania (L.) infantum chagasi* ISOLATED FROM NON-ULCERATED CUTANEOUS LEISHMANIASIS

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In Honduras, infection by *L.* (*L.*) *infantum chagasi* causes, in addition to visceral leishmaniasis (VL), non-ulcerated or atypical cutaneous leishmaniasis (NUCL), in the same geographic area. To better understand the role of the parasite in the pathogenesis of infection caused by *L.* (*L.*) *infantum chagasi* in Central America, the present study evaluated the *in vitro* and *in vivo* host-parasite interaction using isolates from patients with VL and

NUCL of Amapala municipality, Valle, Honduras. For *in vitro* studies, peritoneal macrophages of hamsters were infected, with promastigotes isolated from NUCL (n=4) and VL (n=1), previously characterized. The cultures were incubated at 32 °C, 34 °C, and 36 °C for 24 and 48 hours. We determined the infection index in round coverslips stained by Giemsa; and the supernatants were used for hydrogen peroxide, nitric oxide, and cytokines determination. For *in vivo* studies, we used 10^7 promastigotes isolated from NUCL (n=2) and VL (n=1) to infect hamsters by the intraperitoneal and subcutaneous route. After 15, 30, 60, 90, and 120 days PI, animals were euthanized and biopsies from the skin, liver, and spleen were collected for determination of parasite load by limiting dilution, cytokines by RT-PCR, and histopathological changes. Sera were also collected for determination of IgG levels. *In vitro* study showed a higher macrophage infection index for NUCL strains at 32 °C and 34 °C without difference between 24- and 48-hours PI. However, for VL strain, a higher infection index was observed at 34 °C and 36 °C, especially at 48-hour PI at 36 °C. Low levels of oxygen and nitrogen-derived metabolites, and inflammatory cytokines, were detected in the cultures supernatants of macrophages infected with the different strains, that did not show a correlation with the infection index. In the *in vivo* study, the skin inoculation site did not develop macroscopic lesion, and the histopathological changes were slight for all parasite strains. In the liver, peri-portal mononuclear infiltrate and inflammatory nodules in the parenchyma were observed. The parasite load increased with the time of infection in the animals infected with the dermotropic strains, regardless of the inoculation route, in the liver. Proliferation of macrophages in the splenic red pulp increased with the time of infection, the same way in all experimental conditions; and the parasite load increased with the time of infection when dermotropic strains were inoculated intraperitoneally; and also, when dermotropic and viscerotropic strains were inoculated subcutaneously. The humoral immunity, evaluated by ELISA in the sera of hamsters showed increase of total IgG titers with the time of infection, regardless of the strain and inoculation route used. Concerning cytokines, no expressive increase or decrease in pro and anti-inflammatory cytokines was observed, except for IL-10, that was more evident in all experimental groups. The results showed differences *in vitro* but not *in vivo* studies between dermortropic and viscerotropic strains.

Probably, the complexity of the *in vivo* immune response evolving elements of innate and acquired immunity must interfere in the host-parasite relationship favoring the establishment of *L*. (*L*.) *infantum chagasi* infection in hamster.

Keywords NON-ULCERATED CUTANEOUS LEISHMANIASIS; *L.* (*L.*) *infantum chagasi*; EXPERIMENTAL INFECTION; IMMUNE RESPONSE

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P1-053: THE ESCRT PROTEIN VPS36 IS A CRITICAL COMPONENT OF *LEISHMANIA MAJOR* VESICLE RELEASE AND INFECTIOUS CAPABILITY

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Leishmania is a protozoan parasite that infects mammalian macrophage cells by evading innate and adaptive immune responses. Our lab has shown that small extracellular vesicles (sEVs) released by *Leishmania* within its sandfly vector increase infection severity when co-injected with *Leishmania* in mouse footpads. To assess the impact of inhibiting sEV production in *Leishmania*, the gene Vps36, an important member of the ESCRTII complex key to sEV production in eukaryotic cells, was disrupted using CRISPR/Cas9. A puromycin resistance gene was inserted into the Vps36 exon of *L. major* to generate a Vps36null strain. The Vps36 exon was then restored using plasmid recombination to generate a Vps36addback strain. EVs were collected from cultured parasites using differential ultracentrifugation. EVs were analyzed using nanoparticle tracking analysis (NTA), Transmission Electron Microscopy (TEM), RNAseq, and LC-MS/MS proteomic analysis. Balb/C mice footpads were injected with WT and Vps36null *L. major* parasite either alone or supplemented with purified WT *L. major* sEVs and skin hyperinflammation was monitored. TEM imaging showed that both WT and Vps36null *L. major* released EVs after 37°C temperature shock (TS) and during growth, though there was greater morphological variability in the Vps36null EVs. NTA results suggest Vps36null *L. major* parasites produce significantly less sEVs after TS and during growth. The Vps36addback strain of *L. major* sEV release was like the WT. Vps36null *L. major* sEVs have a unique protein profile compared to WT *L. major* sEVs and this difference was only observed in parasite sEVs and not the whole parasite. Vps36null *L. major* failed to induce a high level of infection in susceptible Balb/C mice

even when co-injected with WT *L. major* sEVs compared to severe infection caused by WT *L. major*. Our results together strongly suggest that the disruption of Vps36 in *L. major* is critically impacting its sEV production. Although vesicle release still occurs in the Vps36null *L. major*, there is a significant reduction in sEV release, which is generally believed to play a greater role in cell-cell communication. This reduction can be directly attributed to Vps36 disruption since Vps36addback is sufficient to restore sEV release. There is also a significant reduction in metabolism-related proteins present in the Vps36null sEVs relative to the WT. This suggests that Vps36 disruption also specifically alters the cargo of *L. major* sEVs and this combined with the overall impaired sEV release can explain the drastic reduction in Vps36null infectivity. Strikingly, the addition of WT *L. major* sEVs did not affect Vps36null *L. major* infection at all whereas it exacerbated the WT *L. major* infection as expected. This suggests that sEVs not only impact initial *Leishmania* infection but continued sEV release is also necessary to maintain infection. Vps36 plays a key role in sEV production by *L. major* and interferes with protein packaging, resulting in a severe reduction in infectious capability.

Keywords VPS36; EXTRACELLULAR VESICLES; INFECTIOUS DISEASE; *Leishmania*; CRISPR

Financing Canadian Institute of Health Research Project Grants

P1-054: SEVERE GENETIC IRON OVERLOAD HAMPERS DEVELOPMENT OF CUTANEOUS LEISHMANIASIS IN MOUSE FOOTPADS

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Leishmania sp. are a group of intra-macrophage protozoan parasites which rely on the essential micro-nutrient iron for survival. Iron plays key roles in many of the parasite's metabolic pathways including electron transport and antioxidant defense. As with most organisms, iron deprivation can stunt growth, whereas iron overload can cause cell death through oxidative stress. As such, proper access and regulation of iron is vital for parasite growth and virulence. Macrophages, the host cell for *Leishmania*, may divert iron flux by reducing expression of iron importers or by increasing expression of the sole iron exporter ferroportin at the cell surface. *Leishmania* sp. have adapted mechanisms to acquire iron despite these cellular adaptations. The predominant physiologically relevant form of iron for parasite development and how conditions of severe iron dysregulation affect parasite growth remain unclear. To understand the role of cellular iron in *Leishmania* infection, we employed mice deficient in hemojuvelin on a C57BL/6 background ($Hjv^{-/-}$) which recapitulates the severe genetic iron loading disorder hereditary hemochromatosis. Lack of this Bmp co-receptor severely limits the production of hepcidin, the iron peptide hormone which induces the degradation of the iron exporter ferroportin at the cell surface. Thus, impaired production of hepcidin leads to massive systemic iron overload due to unrestricted iron export in enterocytes with paradoxical iron deficiency within macrophages which fail to retain or store iron. $Hjv^{-/-}$ mice were injected with *Leishmania major* either in hind footpads and followed for up to 7 weeks or intraperitoneally before collection of peritoneal lavages after 6 hours. Mice were also injected with *Leishmania*

infantum and followed for up to 3 weeks to study visceral leishmaniasis. Hjv$^{-/-}$ mice displayed delayed growth of *L. major* in hind footpads when compared to wild type controls with a significant difference in parasite burden observed at 4 weeks of infection. Popliteal lymph node analysis demonstrated a decrease in the iron transporter *Tfrc* (p=0.06) in infected Hjv$^{-/-}$ mice suggesting an iron elevated environment for the parasite despite lack of macrophage iron stores. Interestingly, following acute intraperitoneal exposure to *L. major*, Hjv$^{-/-}$ peritoneal macrophages had elevated transcription of the cytokines and chemokines *Tnfα*, *Il1β*, *Ccl2*, *Cxcl2* as well as increased IL6 protein as measured by multiplex assay possibly explaining the delay in parasite growth observed in footpads at early timepoints. Yet, Hjv$^{-/-}$ mice infected with *L. infantum* to study visceral pathology showed no difference in parasite burden at 1-, 2-, or 3-weeks post-infection in either liver or spleen, despite the latter having reduced iron content matching previously published data using a model of mild iron overload. Taken together, these results shed light on the role of iron and hemojuvelin at the different stages of infection of *Leishmania* with a greater impact of iron at early timepoints of infection particularly for cutaneous leishmaniasis. Understanding the role of iron in parasite metabolism may help us develop novel treatments and understand the effects of dietary iron supplementation for iron deficiency anemia in parasite endemic regions.

Keywords IRON; *Leishmania*; MACROPHAGES; HEMOJUVELIN; INFLAMMATION

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P1-055: MICRORNAS COOPERATIVELY CONTRIBUTE TO HUMAN MACROPHAGE SUSCEPTIBILITY TO *L. amazonensis* INFECTION THROUGH METABOLIC REPROGRAMMING

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Leishmania amazonensis is a parasitic protozoan causing mainly cutaneous leishmaniasis in humans. Macrophage undergoes metabolic reprogramming as the primary host cell of *Leishmania* by extensive modulation of gene expression at early infection. Previous studies demonstrated posttranscriptional modulation of macrophage function by microRNAs (miRNAs). As a multilevel process, miRNAs target messenger RNAs (mRNAs) and proteins culminating in metabolite changes that favor the parasite's survival and replication within the hostile phagolysosome environment. We performed RT-qPCR for 84 microRNAs (miRNAs or miR) related to the immune response of *L. amazonensis* early infected (4 and 24 hours) human THP-1 derived macrophages. We found that the upregulated miRNAs miR-372, miR-373, and miR-520d comprise a miRNA family since they share the seed sequence, nucleotides from positions 2-8, responsible for target mRNA recognition. These miRNAs are also upregulated in data from our collaborators regarding *L. braziliensis* and *L. donovani* infection in THP-1 and serum from *L. braziliensis*-infected patients. The seed sequence is also present in the homologous murine miRNA mmu-miR-294 that targets the nitric oxide synthase 2 mRNA. Thus, upregulation of miRNAs containing the seed sequence AAGUGCU is a widespread mechanism regardless of the host and *Leishmania* species and is consistent with *in vitro* and *in vivo* findings in the literature. On the other hand, the target mRNA is not conserved, as we found the 3'UTR site of NOS2 to be mutated in humans. We performed *in silico* analysis to predict which pathways could be controlled by the miR-372, miR-373, and miR-520d in humans. Our findings show that

glucose transporters such as GLUT1 (SLC2A1), GLUT3, and GLUT6 are enriched among the predicted targets. Further investigation showed other putative immunometabolic targets, such as the cationic amino acid transporter SLC7A2 (CAT2), hexokinase 1 (HK1) and the transcription factor HIF-1α. Given that both arginine and glucose metabolism are essential for macrophages' response, the identified miRNAs are candidates to modulate infection susceptibility. To elucidate their impact on human macrophages, we inhibited miR-372, miR-373, and miR-520d with antisense oligonucleotides either alone or in combination. We observed that, by inhibiting miR-372, but not miR-373 or miR-520d alone, the proportion of infected macrophages and the number of amastigotes per macrophage is reduced. Also, simultaneously inhibiting miR-372, miR-373 and miR-520d further decreased infectivity, indicating cooperative action impairing early infectivity. The simultaneous inhibition of miRNAs also leads to an increase of GLUT1 and CAT2 mRNA amount, showing that miR-372, miR-373, and miR-520d can target both glucose and arginine transporters. Metabolomic profiling of THP-1 during infection under miR-372 family showed that miRNAs can interfere with macrophage metabolic reprogramming by reducing polyamines availability. In conclusion, we reported a microRNA family upregulated in *L. amazonensis* infected THP-1 macrophage correlated with infection susceptibility by regulating polyamine content in macrophages. These findings are relevant because miRNAs, either alone or in combination, are already being used in clinical studies for anti-tumor therapies. Given the current status of increasing drug resistance, *Leishmania* researchers are looking for potential targets for host-directed therapy.

Keywords MIRNA; POSTTRANSCRIPTIONAL REGULATION; GENE EXPRESSION METABOLISM; METABOLOMICS

Financing Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)

P1-056: THYMIC ALTERATIONS RESULTING FROM EXPERIMENTAL VISCERAL LEISHMANIASIS IN SIRIAN HAMSTER *(Mesocricetus auratus)*

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The thymus is a lymphoid organ responsible for the development and maturation of T cells, which make up the Th1, Th2, Th17 and Treg immune responses, involved in the response to visceral leishmaniasis. For the maturation and immunological development of T lymphocytes, a bidirectional interaction between the thymic microenvironment, composed of epithelial cells, dendritic cells, macrophages and the extracellular matrix with the differentiating lymphocytes is necessary. In this study we evaluated the morphological characteristics and tissue distribution of hematopoietic and stromal cells in the thymus of hamsters experimentally infected with *Leishmania infantum* in order to identify changes that may contribute to the understanding of the pathophysiology of the disease. For this purpose, 15 hamsters experimentally infected with 10^7 promastigotes of *L. infantum* (MHOM/BR/1972/BH46) were inoculated intraperitoneally, and 5 of them were euthanized at 15-, 60- and 120-days PI. Fifteen healthy hamsters were used as control and 5 of them were also-euthanized at 15, 60 and 120 days. Histological sections of thymus stained with hematoxylin and eosin were evaluated, and immunohistochemistry was performed to determine the cellular composition for labeling mesenchymal cells (vimentin+), epithelial cells (cytokeratin+), macrophages (MAC387+), B lymphocytes (CD79a+) and T lymphocytes. (CD3+). To evaluate the immunostaining pattern, we considered its presence or absence, the characteristic of cell morphology

and the distribution in the thymic parenchyma. The percentage of labeled cells was estimated by the average of positive cells in 10 different images by histological sections captured by computerized image analysis system in 40 objective. The images were analyzed using the ImageJ 1.52a software. Immunohistochemistry was also used to detect parasite amastigotes in the thymus. Statistical differences were determined by ANOVA and Tukey test. The thymic morphology and the degree of atrophy were not different between the infected and controls hamsters, this absence of significant atrophy in the thymus of the infected animals may have been due to the chronic nature of VL, with thymic atrophy generally described as a result of acute infections. After 15 days of infection, there was an increase in vimentin+ cells and CD3+ T lymphocytes in the thymus, which decrease with time of infection to normal parameters values. The increase in the number of T lymphocytes in the thymus followed by normalization with the chronicity of the infection has already been described in *Trypanosoma cruzi* infection. The number of CD79a+ B lymphocytes after 120 days of infection were reduced in relation to the control, which can be explained by the recruitment of B lymphocytes due to the Th2 response observed in progressive visceral leishmaniasis. Furthermore, *L. infantum* amastigotes were present in the thymus of 9/15 hamsters, in the medullary and corticomedullary regions. The presence of the parasite would interfere with the dynamics of differentiation process, since the microenvironment necessary for the development of T lymphocytes is maintained by a bidirectional interaction and changes in thymic cellular population were observed.

Keywords LEISHMANIASIS; IMMUNOHISTOCHEMISTRY; HISTOPATHOLOGICAL CHANGES

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P1-057: IMMUNOHISTOCHEMICAL INVESTIGATION OF INFLAMMATORY CHANGES IN THE BRAIN OF HAMSTERS INFECTED WITH *Leishmania Infantum*

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Visceral leishmaniasis (VL) is a chronic disease with systemic changes and progressive character that affects dogs and humans in a similar way. In dogs, visceral leishmaniasis (CanL) causes a variety of systemic clinical manifestations, which may include neurological changes, such as generalized seizures, cranial nerve palsy, tetraparesis and quadriplegia; however, even in the absence of clinical neurological symptoms, histopathological changes such as leptomeningitis, choroiditis and vascular congestion may be present in the brain. Few studies report the occurrence and pathogenesis of brain lesions in cases of natural or experimental *Leishmania infantum* infection in dogs and human beings. The present study aimed to describe the cellular and morphological changes found in the brain of hamsters inoculated with *Leishmania infantum* (MHOM/BR/1972/BH46). Fifteen Syrian hamsters (*Mesocricetus auratus*) infected with *L. infantum* and nine not infected (control) were euthanized at different times (30, 60 and 120 days). Morphological investigation of the brain was performed by staining the 5-µm sections with haematoxylin-eosin (HE) and immunohistochemistry for detection of inflammatory T lymphocytes (CD3+), astrocytes (GFAP+) and microglia (IBA1+). The result of immunohistochemistry was given by the average number of CD3+ cells manually counted, and the average percentage of area staining for GFAP and

IBA1 evaluated with the ImageJ program. For that, 10 random images of each area of the brain (cortex, meninges and choroid plexus) of each animal were captured with a microscope coupled to a camera and computer. In the histopathological (HE) evaluation, it was possible to observe a predominant mononuclear inflammatory infiltrate, with discrete intensity and localized mainly in the meninges, and we did not find any amastigote forms. Regarding immunohistochemistry, we observed an increase in the number of TCD3+ lymphocytes in the meninges and choroid plexus at 120- compared to 30- and 60-days PI ($p<0.05$). The percentage of GFAP and IBA1-labeled areas in the cortex also increased with the evolution of the infection. With a higher average of area marked with GFAP in groups 60 and 120-days PI in relation to the control ($p<0.05$), and also with higher area with IBA1 after 120-days PI in relation to the control and 30-days PI. The increase in of the number of TCD3+ cells, together with the increased intensity of immunostaining for astrocytes and microglia suggest activation of the inflammatory response and a break of balance in brain homeostasis. It does not depend on the presence of parasite and occurs possibly in response to systemic inflammation. The harmful or beneficial effects of astrogliosis and reactive microgliosis on the brain of during *L. infantum* infection are poorly known and still need to be further investigated.

Keywords INFLAMMATION; NERVOUS TISSUE; LEISHMANIASIS; GLIAL CCELL

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P1-059: AN INSIGHT INTO THE PROTEIN CONTENT OF THE EXOSOMES ISOLATED FROM *Leishmania infantum* AMASTIGOTES AND *IN VITRO*-INFECTED HUMAN PHAGOCYTES

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Exosomes are ~100 nm extracellular vesicles of endocytic origin. Cells produce exosomes as a mechanism of intercellular communication. These vesicles may also have excretory functions. Exosomes contain lipids, including their lipid bilayer, proteins, mRNA, and micro-RNA. This study aims to identify proteins in exosomes from amastigotes of the *Leishmania infantum* IPER/ES/2013/ATE1FL6 isolate and *in vitro* infected phagocytic cells from the U937 human myeloid cell line stimulated with phorbol esters. Said *L. infantum* isolate is associated with the Fuenlabrada (Community of Madrid, Spain) active outbreak and was obtained from *Phlebotomus perniciosus* specimens captured in this area. Exosomes were isolated from concentrated supernatant samples by size exclusion chromatography. The intracellular content of infected U937 cells was also processed using a 0.1% SDS mild lysis procedure. The eluate fraction containing the higher extracellular vesicle density distributed around 100 nm in diameter was selected from each extracellular vesicle sample by nanoparticle tracking analysis (NTA). Total protein extracts were prepared from these fractions and proteins were identified by LC-MS/MS using a Q Exactive System (Thermo Scientific). Mass spectra were processed with Proteome Discoverer 1.4.1.14 (Thermo Scientific) and proteins were identified by SEQUEST using the *L. infantum* JPCM5 (TriTrypDB) and the human (Uniprot) genome sequences. Exosomes from amastigotes contain the gp63 virulence factor and the heat shock proteins hsp70 and hsp83. These molecules are parasite exosome markers. The tacrolimus (FK506)-binding

protein (FKBP), the elongation factor 1α, the 3-hydroxymethylglutaryl-CoA synthase, the 4-coumarate-CoA ligase, and the hsp60 chaperonin are also present in amastigote exosomes. The infected phagocyte secretes exosomes that contain apoptosis (e.g. annexin V, caspases), cell adhesion (e.g. integrins, desmoplakin), and immune system cellular marker (e.g. HLA I-related proteins, lectins) proteins. A mitochondrial hsp70 protein is also present in phagocyte exosomes. These results suggest that the exosomes of infected host cells contain signaling molecules related with the immune response in a context of parasite adaptation and host cell degradation.

Keywords *Leishmania infantum*; U937 MYELOID HUMAN CELL LINE; EXOSOMES; PROTEIN CONTENT

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P1-060: UNDERNUTRITION ALTERS EXPRESSION OF SPLENIC EXTRACELLULAR MATRIX COMPONENTS AND MODIFIES SPLENOCYTES LOCATION IN MICE INFECTED WITH *Leishmania infantum*

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Undernutrition is a risk factor for the development of visceral leishmaniasis (VL). Our group demonstrated that protein undernutrition alters the number of splenocytes, the microarchitecture and the immune response in the spleen of infected animals, suggesting that the distribution and localization of T and B lymphocytes in the organ, as well as the expression of molecules involved in cell migration may be compromised. To assess this, we analyzed the distribution, location and activation of subpopulations of lymphocytes in the spleen of undernourished mice infected with *Leishmania infantum*, as well as the expression of extracellular matrix (ECM) molecules in this organ. The animals were fed a control diet (CP - 14% protein) or a low protein diet (LP - 4% protein). After seven days of diet, each group was divided into two and one of them was infected with *L. infantum*, resulting in four experimental groups: CP, LP, CPi and LPi. After 14 days of infection, the animals were euthanized and the spleen was analyzed. Immunohistochemical analysis revealed increased expression and deposition of laminin and fibronectin in the splenic red pulp of LPi animals. Furthermore, MMP-10 levels were increased in the spleen of LP and LPi

mice, whereas TIMP1 levels were decreased in these animals, suggesting that the splenic disorganization induced by undernutrition is mediated by changes in ECM. Since tissue organization drives cells location and this in turn determines their correct activation, we evaluated whether undernutrition would alter location, number and proliferation of T and B lymphocytes. We observed hypertrophy of the periarteriolar lymphatic sheath mediated by the accumulation of $CD4^+$ T cells and a significant increase in these cells in the follicular region of LP and LPi animals. Furthermore, we observed a significant increase in $CD8^+$ T cells in the red pulp of LPi animals. While CPi animals show a significant decrease in $B220^+$ cells, LPi animals show a significant increase in the percentage of positive area for this marker in the splenic red pulp. We also observed a significant decrease in the cell proliferation profile in the spleen of LP and LPi mice and a significant and early increase in the splenic parasite load of LPi animals. Together, our results suggest that splenic disorganization induced and aggravated by undernutrition compromises T-cell mediated control of parasites in this organ.

Keywords PROTEIN UNDERNUTRITION; *Leishmania infantum*; SPLEEN; LYMPHOCYTE SUBPOPULATIONS; EXTRACELLULAR MATRIX (ECM)

Financing FAPERJ, CAPES, FIOCRUZ, CNPq

P1-061: HIF-1α STABILIZATION IN HUMAN MACROPHAGES DURING LEISHMANIA MAJOR INFECTION IS IMPAIRED BY PARASITE VIRULENCE.

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Hypoxia-inducible factor-1 alpha (HIF-1α) is described as a master regulator of immune and metabolic cell functions. As a transcriptional factor whose activity is directly correlated to oxygen levels, HIF-1α is a tempting target to understand infectious disease control. *Leishmania major* parasites are known to readily subvert host macrophage functions for their survival and induce local oxygen consumption at the site of infection.
Several studies, including ours, demonstrate that HIF-1α could be one of the major players in this infectious process. However, its role has been studied using long term established cultured parasites from different *Leishmania* species, but never in relation to parasite virulence. In this work, we studied the expression levels of HIF-1α and the related VEGF-A angiogenic factor under its control in human macrophages infected with promastigotes from two well characterized *Leishmania major* human isolates classified as hypo- or hyper-virulent. Our results show that, unlike hypo-virulent parasites, which induce high HIF-1α expression levels, either under normoxic or hypoxic conditions, hyper-virulent *Leishmania major* parasites are capable of reducing macrophagic HIF-1α expression, and consequently impede the expression of VEGF-A mRNA. The fact that *Leishmania* virulence might impairs HIF-1α expression, further reinforces this transcription factor as a key player in the mechanisms controlling disease chronicity, severity or outcome.

Keywords *Leishmania major,* MACROPHAGE, HIF-1α, VEGF-A, HYPOXIA, VIRULENCE

P1-062: PARTICIPATION OF PD-1/PD-L1 IN SUSCEPTIBILITY TO *LEISHMANIA AMAZONENSIS* INFECTION AND INDUCTION OF PD-L1 IN NEUTROPHILS *IN VITRO* AND *IN VIVO.*

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Neutrophils (NΦ*s*) play an important role in the context of leishmaniasis, contributing to exacerbate or control the progression of the infection, a dual effect whose underlying mechanisms are unclear. Given that the PD-1/PD-L1 interaction can promote cellular dysfunction, and that NΦs can interact with T cells during infection, we investigate the levels of PD-L1 in NΦs exposed to *Leishmania*. We demonstrated that both *L. amazonensis* promastigotes and amastigotes induced the expression of PD-L1 in human and murine NΦs that internalized these parasites *in vitro*. PD-L1-expressing NΦs were identified in the ear lesions and in the draining lymph nodes of *L. amazonensis*-infected mice, assessed through cell cytometry and intravital microscopy. Moreover, PD-L1 expression increased progressively in NΦs of ear lesions as the disease progressed to the chronic phase. Importantly, PD-L1^{+} NΦs were detected in the lesions of patients with cutaneous leishmaniasis. Together, these findings suggest that *Leishmania* increases the expression of PD-L1 in NΦs, which could favor the parasite's survival. We also evaluated the expression of PD-1 in lymphocytes and of PD-L1 in antigen-presenting cells, a role still little known in cutaneous leishmaniasis. The roles of PD-1 in lymphocytes and PD-L1 in antigen presenting cells have been well studied in tumors and other models of infection; but little is known about their roles in cutaneous leishmaniasis. Seeking to understand the importance of the PD-1/PD-L1 pair, we evaluated the therapeutic potential of anti-PD-1 and anti-PD-L1 monoclonal antibodies against *L. amazonensis* infection in BALB/c mice.Our results showed that treatments

with anti-PD-1 and anti-PD-L1 significantly increased IFN-ɤ production in $CD4^+$ and $CD8^+$ T cells. Mice treated with anti-PD-1 and anti-PD-L1 exhibited larger lesions and significantly lower parasitic loads in relation to controls.The treatment did not affect the production of anti-*Leishmania* antibodies or IL-10, but the anti-PD-1 treatment reduced the production of IL-4 and TGF-β. Together, our results highlight the therapeutic potential of treatment with anti-PD-1 and anti-PD-L1 in reinvigorating T cells to control parasitic burden and the participation of the pair PD-1/PD-L1 in susceptibility to infection.

Keywords *Leishmania amazonensis*; NEUTROPHIL; IMMUNOTHERAPY; PD-L1; PD-1

P1-063: CHARACTERIZATION OF *LEISHMANIA (L.) AMAZONENSIS* OLIGOPEPTIDASE B AND ITS ROLE IN MACROPHAGE INFECTION

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Leishmania spp. are parasitic protozoa that cause leishmaniasis, an endemic disease in more than 98 countries, with more than 1 million new cases every year in the world. Symptomatic infection in man manifests itself in different clinical forms, which are grouped into cutaneous and visceral. *Leishmania* promastigotes are transmitted by the vector and differentiate into amastigotes within the phagocytic cells of the vertebrate host. To survive the varied and hostile environments, the parasite has several virulence factors. Oligopeptidase B (OPB) is a serine peptidase of the prolyl peptidase family (clan SC, family S9) present in prokaryotes, some eukaryotes and some higher plants. This protein has been considered a virulence factor in trypanosomatids, but only a few studies have analyzed its role in *Leishmania* virulence or infectivity, all of them with Old World parasite species. OPB-deficient *Leishmania (Leishmania) major* has impaired differentiation into metacyclic promastigotes, is less infective in macrophages *in vitro* and generate smaller footpad lesions in mice. *L. (L.) donovani* promastigotes knockout (ko) for OPB had more isoforms of enolase and higher abundance of this enzyme on the surface. In this work we produced recombinant OPB from *L. (L.) amazonensis*, an important agent of leishmaniasis in Brazil. We showed that its pH preferences and inhibition patterns are similar to those reported for *L. (L.) major* and *L. (L.) donovani* enzymes. Since *Leishmania* is known to secrete OPB, we performed *in vitro* infection assays using the recombinant enzyme. Our results show that active soluble OPB increases *in vitro* infection by *L. (L.) amazonensis* when present before and throughout

infection. Further studies should be performed to better understand these findings.

Keywords OLIGOPEPTIDASE B; ENZYME ACTIVITY; INHIBITION; MACROPHAGE INFECTION

Financing FAPESP; CAPES.

P1-064: STUDY OF THE INFECTIVITY AND ADVERSE EFFECTS OF *LEISHMANIA AMAZONENSIS* ANTIGEN INOCULATED IN HEALTHY MUS MUSCULUS C57BL/6 MICE

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The Montenegro intradermal reaction represents an easy method, with high sensitivity and low cost, which allows its wide dissemination and ensures that it can establish itself as the main diagnostic method in routine exams, in Brazil and in the world, for the detection of cutaneous leishmaniasis. The present study is aimed to analyzes the infectivity and safety of a *Leishmania amazonensis* antigen PH8 (ALA) produced by the Biologic Institute of University of Brasília. Were used 30 isogenic mice, female, *Mus musculus* species, strain C57BL/6. The mice were divided into groups from A to E, with a control group and 4 groups inoculated with 0.1 ml of ALA. The skin reactions in mice 48h after ALA inoculation and the level of biochemical markers did not show important changes compared to the control group and with the reference values, as well as the cultures of the lesion aspirate, leishmania did not grow in none of the seeded media. The tested antigen was able to evoke a cellular immune response in mice even at the highest dilutions, which is fundamental for the positivity of the diagnosis through of the intradermal test and did not seem to arouse toxic potential on the animals used with safety in relation to renal and hepatic functions, measured by biochemical markers. One of the inoculated animals had an

amastigote present in the skin biopsy, therefore the antigen preparation must be reviewed and subjected to new safety tests.

Keywords *Leishmania amazonensis* ANTIGEN; MONTENEGRO INTRADERMAL REACTION*;* LEISHMANIASIS DIAGNOSIS*;* CUTANEOUS LEISHMANIASIS; ANTIGEN *L. amazonensis* IINFECTIVIT

P1-066: VIABILITY OF *LEISHMANIA DONOVANI* PROMASTIGOTES: AN EX VIVO STUDY

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Cutaneous Leishmaniasis (CL) caused by *Leishmania donovani* is a growing public health issue in Sri Lanka. The disease is transmitted through the vector sandflies and *Phlebotomus argentipes* is identified as the most probable vector in Sri Lanka. Although the presence of *L.donovani* DNA within wild caught *P.argentipes* has been demonstrated there is lack of evidence to incriminate it as the true vector. The aim of this study was to determine the viability of *L. donovani* parasites reconstituted in blood at 37^{o}C to perform artificial feeding of sand flies, which is the first step to demonstrate evidence for its ability to infect sand flies. Human blood drawn from a healthy donor was immediately mixed with early transformed promastigotes of *L. donovani* (5×10^{6}/ mL) and incubated at 37^{o}C. Cell viability was calculated as the number of viable promastigotes divided by the total number of promastigotes. Thus, number of viable and non-viable promastigotes were counted under the microscope (40x) using trypan blue assay. Percentage of viability remained 100% up to 2 hours after reconstitution in blood while it reduced to 97% at 2 1/2 hours and 3 hours time points. The findings indicate that the parasites remain viable in human blood at 37^{o}C over sufficient durations to enable artificial feeding for subsequent demonstration of infectivity in sand flies.

Keywords *Leishmania donovani*; *PHLEBOTOMUS ARGENTIPES*; VIABILITY; VECTOR INCRIMINATION

P1-067: CONNECTING THE DOTS: A CARTOON NETWORK THAT PEEKS INTO *LEISHMANIA* SPP INFECTION AND EVASION

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Leishmaniosis is a severe zoonotic disease endemic in many countries and affecting millions of humans and animals worldwide. Although macrophages are the definitive host cells, neutrophils are the first cells to encounter the parasite soon after its inoculation in the dermis by the phlebotomine vector, and being competent effector cells are able to control the initial infection. However, parasites can evade intracellular effector mechanisms and be transferred to the definitive host cell, the macrophage. In recent years, it has been demonstrated that host immune response is mainly organ-specific, and the role of other cells in innate immune response, such as hepatocytes have started to emerge. Hepatocytes constitute the majority of hepatic cells and play a key role in controlling innate immunity as well. Also, investigating closely distinct macrophages lineages (MØs - blood macrophages and liver macrophages - KC), it has been demonstrated that the response can shift from immunocompetent actions based on active phagocytic cells with the capacity to destroy pathogens, to tolerogenic

immune reactions, allowing replication and persistence of the parasite inside the host. Forward-looking research approaches such as isolation of extracellular vesicles (EVs) produced by *Leishmania* parasites are also under study and are a focus of our group in order to evaluate the immunogenic potential of EVs. With such a huge quantity of parameters involved in the success or failure of the immune response and persistence of the infection is paramount to get it in simple lines for a multidisciplinary perspective in one health viewpoint. In order to contribute to that we gathered the main results from the last years of research of our group and we present it as scientific illustrations crossed with ultrastructure images. Researchers engaged in investigating biological processes related to zoonotic diseases can contribute with consistency in providing a simple and accurate explanation of complex pathways.

Keywords LEISHMANIOSIS; SCIENTIFIC-ILLUSTRATION; INFECTION-EVASION; IMMUNE-COMPETENT MECHANISM

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P1-068: ALGINATE-COLLAGEN THREE-DIMENSIONAL (3D) CELL CULTURE SYSTEM FOR MODELLING HUMAN *LEISHMANIA* INFECTION

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The earliest step of a natural *Leishmania* infection involves the interaction of promastigotes with components of the dermal tissue, especially the host extracellular matrix (ECM) network. In turn, ECM components can influence immune cell functions, as well as parasite migration and survival. However, most of the models used to investigate the early *Leishmania* host-pathogen interactions are based on two-dimensional (2D) cell culture systems; an evident oversimplification of the initial interactions established between the parasites and the host. Here, we present a multidimensional cell culture system to investigate *Leishmania* host-pathogen interactions. We generated a simple 3D culture system based on an alginate-collagen matrix to encapsulate human peripheral blood mononuclear cells (PBMCs). Encapsulated PBMCs were kept for up to 96 hours with a reduction in cell viability of less than 30%. *Leishmania* infection was conducted post PBMCs encapsulation, aiming to mimic the passage of promastigotes through the extracellular matrix of the host skin tissue. Infection conditions were optimized to achieve a parasite burden similar to those found in skin lesions of cutaneous leishmaniasis patients (3337 ± 587 parasites/1000 PBMCs). We monitored infection using fluorescence microscopy. *Leishmania* promastigotes penetrated the matrix and infected host monocytes overnight. RNA was extracted from de-capsulated infected PBMCs and *ccr2, csf1, il1β, ifn-γ* and *cxcl10* transcripts were evaluated by RT-qPCR and compared with infections in 2D cell cultures. *ccr2, ifn-γ*, and *cxcl10*

showed different trends of gene expression compared with 2D cell cultures 24 and 48 hours post-infection. *csf1* and *il1β* were upregulated in 2D and 3D cell cultures, but the magnitude of modulation was less in 3D cell cultures at 24 and 48 hours post-infection. The 3D cell culture system of alginate-collagen embedded PBMCs is easily adaptable since it does not require specialized equipment and allows for multiparameter readouts. It provides a suitable infection model that resembles *in vivo* characteristics of the *Leishmania* and human host cells interactions and can be applied to evaluate diverse aspects of the infection, immune responses, and pharmacodynamics, features that combined are key to the design of improved drugs and regimens.

Keywords THREE-DIMENSIONAL (3D) CELL CULTURE; DERMIS; EXTRACELLULAR MATRIX; COLLAGEN; MONOCYTES; *Leishmania*-HOST INTERACTION

P1-069: HUMAN NEUTROPHIL ACTIVATION RESPONSE TO INFECTION BY CLINICAL STRAINS OF *L. (V.) braziliensis* HAVING DIVERGENT ANTILESHMANIAL DRUG SUSCEPTIBILITY AND MODULATION BY ANTILEISHMANIAL DRUGS

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Neutrophils are the predominant granulocytes in blood and have a critical role in the control of microbial infections. Recent studies have demonstrated that activation of human neutrophils *ex vivo* is differentially modulated by infection with clinical strains of *Leishmania. (Viannia) panamensis* having intrinsic tolerance/resistance to antimony (SbV) compared to sensitive strains, and by exposure to the antimonial drug in the absence of infection. To determine whether susceptibility to SbV or miltefosine (MIL) of other *(Viannia)* species influenced neutrophil functions, we evaluated the impact of infection by clinical strains of *Leishmania (Viannia) braziliensis* having defined susceptibility phenotypes to anti-leishmanial drugs on neutrophil function. Neutrophils obtained from healthy donors (n = 6) were infected with clinical strains of *L. (V.) braziliensis* that presented susceptibility at the upper (n=10) and lower extremes (n=10) of the range of susceptibility to miltefosine (MIL), and divergent susceptibility to SbV. Infected neutrophils were exposed to clinically relevant drug concentrations: 32 µg SbV/mL and, 4 µM of MIL. The activation profile of neutrophils was evaluated based on the expression of CD66b, CD18, CD62L cell surface markers using flow cytometry, production of reactive oxygen species (ROS) by luminometry, and quantification of Neutrophil Extracellular Trap (NET) formation by PicoGreen fluorescence assay. These activation parameters were analyzed *ex vivo* in relation to infection, parasite susceptibility phenotype, and drug effects. Differences in the expression of activation markers were estimated

using unpaired Student's t-test, and the non-parametric Mann-Whitney test, based on data distribution. Compared with uninfected cells, neutrophils infected with clinical strains of *L. (V.) braziliensis*, displayed an activation profile evidenced by the significant induction of ROS production high expression of CD66b, and reduction of CD62L, but no difference in CD18 expression. Notably, no differences were observed between the activation profiles elicited by infection with sensitive compared with resistant strains for either drug. However, exposure of infected cells to anti-leishmanial drugs modulated neutrophil activation. Following two hours of infection, ROS production was significantly reduced by exposure to MIL, while SbV did not affect ROS production. Conversely, NET formation was diminished in the presence of SbV but not by exposure to MIL. Exposure to SbV induced an increase in neutrophil activation demonstrated by the higher expression of CD66b and reduced expression of CD62L whereas exposure to MIL did not modulate expression of these markers. In conclusion, clinical strains of *L. (V.) braziliensis* similarly induced the activation of neutrophils independently of their susceptibility phenotype for anti-leishmanials drugs. However, MIL and SbV distinctly modulated the activation profile of infected neutrophils.

Keywords NEUTROPHILS; CLINICAL STRAINS; DRUG RESISTANCE; ANTIMONY; MILTEFOSINE

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P2-053: IMMUNE RESPONSE PROFILES FROM HUMANS EXPERIMENTALLY EXPOSED TO *P. duboscqi* BITES

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Cutaneous leishmaniasis is a vector-borne neglected infectious disease prevalent in 92 countries with approximately one million infections annually. Interactions between vector saliva and the human host alter the response to infection and outcome of disease caused by *Leishmania major*. To understand the human immunological responses developed against saliva of the *L. major* vector, *Phlebotomus duboscqi* (Pd), we repeatedly exposed the arms of 14 healthy U.S. Pd-seronegative volunteers to 10 uninfected laboratory-reared Pd with up to nine sessions/year (Sand Fly Feeding [SFF]1 to SFF9). Specific anti-Pd salivary gland homogenate (SGH) IgG antibodies were determined by ELISA and Western blotting (WB). Peripheral blood mononuclear cells (PBMCs) were cultured in the presence of SGH or recombinant proteins and supernatants collected after 96h for IFN-γ release assay. After the last Pd exposure session, a punch skin biopsy from the bite site of some volunteers was performed 48h after bites. The biopsy was used for histological staining for various markers and RNA extraction (RNA easy fibrous tissue kit) for transcriptome analysis. Using mRNAs from two sandflies salivary glands, PCR-based cDNA libraries were

created, sequenced and analyzed. A variety of immediate site reactions with punctuate lesions and erythematous patches (SFF1 to SFF5) and induration (SFF7 to SFF9) were observed. Late reactions were also observed with macular discoloration/pigmentation/erythematous papules (SFF1 to SFF3) being the most common. Reactivation of prior contralateral arm bite sites with subsequent sand fly bite exposures was noted. One participant developed a severe wheal and flare/urticarial reaction. Hematoxylin and eosin staining of bite site skin biopsy sections showed mainly moderate mononuclear infiltrate with occasional eosinophils. Immunohistochemistry analysis demonstrated a varied mild to moderate cellular infiltrate with CD3 T lymphocytes (CD3+ CD20-), macrophages (CD68+), neutrophils (Myeloperoxidase) and some eosinophils (Luna). Human RNA sequencing of these specimens revealed expression profile differences between control skin and the Pd bite site. An over-expression of genes in pathways such as leucocyte activation, lymphocyte aggregation and regulation of immune response was observed in the bite site while epithelium and tissue development, as well as lipid metabolic process pathways, were under-expressed in the skin of the bite site when compared to control. Modest increases in plasma antigen-specific IgG responses to SGH were seen over time. WB results showed volunteer plasma reactivity to Pd salivary proteins (~ 14-100kDa). Additionally, WB indicated some immunodominant proteins (~ 62, 44, 32, 20 and 15kDa) which may be associated with the number of Pd exposures showing higher IgG reactivity in later sand fly exposures. Preliminary data demonstrated that some volunteers developed a cellular immunity exhibited by the secretion of IFN-γ upon PBMC stimulation with Pd SGH and saliva recombinant antigens. In conclusion, humans make a local (skin) and systemic immune response against Pd salivary proteins indicating that the further development of salivary recombinant proteins represents a crucial approach for future development of a potential *Leishmania* vaccine.

Keywords *P. duboscqi*; SALIVA; ANTIGEN; VACCINE; IMMUNE RESPONSE

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P2-054: INVOLVIMENT OF DENDRITIC CELLS IN THE SKIN LESION OF NON-ULCERATED CUTANEOUS LEISHMANIASIS CAUSED BY *Leishmania* (*L.*) *infantum chagasi*

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Human *Leishmania* infection causes a spectrum of clinical and immunopathological manifestations, depending on the parasite species and the genetic-immunological host background. *Leishmania* parasites are obligate intracellular parasites, so the first steps after infection are crucial for determination of the infection. In this sense, to better understand the pathogenesis of non-ulcerated cutaneous leishmaniasis (NUCL) caused by *L.* (*L.*) *infantum chagasi* in south region of Honduras, the present study evaluated the involvement of antigen-presenting cells (APC) subpopulations in skin lesions of patients affected by NUCL using cellular and intracellular markers by double-stained immunohistochemistry. Ten skin biopsies from patients affected by NUCL with a positive direct parasitological diagnosis, that was confirmed and characterized by *hsp*70 PCR-RFLP, were used. Histological sections were used for histopathological study and immunohistochemistry reaction. Paraffin sections stained by Hematoxylin-Eosin were used for histopathological analysis and double-stained immunohistochemistry was used to characterize Langerhans cells

(CD1a/IL-10, CD1a/IL-12, CD207/IL-10, CD207/IL-12) and dermal dendritic cells (CD11c/IL-12 and CD11c/IL-10) subpopulations. The chromogens DAB+H2O2, Permanent Red, and Esmerald Chromogen were used to reveal the reaction. The immunolabeled cells were quantified considering the pattern of staining as well as cell morphology. The cellular density (the number of cells per square millimeter) of each marker was determined by the ratio between the immunostained cells and the area of each photo. The *hsp*70 PCR-RFLP using the restriction enzyme *Hae*III characterized the parasites in the NUCL biopsies as *L.* (*L.*) *infantum chagasi*. Microscopically, slight morphological changes were observed in the epidermis; by the other hand, more evident changes were present in the dermis, which was characterized by lymphohistiocytic inflammatory infiltrate varying between moderate and intense with diffuse arrangement and associated with the epithelioid granulomas and discreet parasitism. Immunohistochemical analysis showed the presence of all markers used in this study. Quantitative analysis of the double-staining immunohistochemistry showed a density (average ± standard error) of 239.7 ±17.7 for $CD1a^+/IL\text{-}12^+$ cells, 58.1 ± 16.1 for $CD1a^+/IL\text{-}10^+$ cells, 243.0 ± 21.9 for $CD207^+/IL\text{-}12^+$ cells, 37.9 ± 5.2 for $CD207^+/IL\text{-}10^+$ cells, 424.9 ± 37.6 for $CD11c^+/IL\text{-}12^+$ and 130.2 ± 20.4 $CD11c^+/IL\text{-}10^+$ cells. The results showed higher number of Langerhans cells (CD1a, CD207) and dermal dendritic cells (CD11c) producing pro-inflammatory (IL-12) than anti-inflammatory (IL-10) cytokines in the skin lesions of patients affected by NUCL ($p < 0.05$) suggesting that these subpopulations of APC could be drive the host immunity to the resistance pole to *L. (L.) infantum chagasi* infection helping the development of Th1 and/or Tc1 cellular immune response.

Keywords *L.* (*L.*) *infantum chagasi;* NON-ULCERATED CUTANEOUS LEISHMANIASIS; IMMUNOHISTOCHEMISTRY; APC

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P2-055: *IN SITU* EVALUATION OF CD4 AND CD8 CELLULAR IMMUNE RESPONSE IN NON-ULCERATED CUTANEOUS LEISHMANIASIS CAUSED BY *Leishmania (L.) infantum chagasi*

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In leishmaniasis, the process of parasite killing requires the differentiation of CD4 and CD8 T lymphocytes subpopulations. The ability of these cells to contribute to protective or pathological mechanisms is directly related to their effector functions and cytokine production. In this sense, the present study aimed to characterize the type of *in situ* cellular immune response in the lesions of patients affected with rare and atypical cutaneous leishmaniasis caused by *Leishmania (L.) infantum chagasi* in Honduras, Central America through cellular and intracellular markers by double-stained immunohistochemistry. We used ten skin lesion biopsies from patients affected by non-ulcerated cutaneous leishmaniasis (NUCL) with previous parasitological diagnosis by direct exam, which was confirmed by *hsp*70 PCR-RFLP. Histological sections of NUCL skin lesions were used for immunohistochemical reaction using primary antibodies to CD4 and CD8 T lymphocytes, and IFN-ɣ and IL-10 cytokines. To evaluate the lymphocytes subpopulation, double-stained immunohistochemistry using CD4/IFN-γ,

CD4/IL-10, CD8/IFN-ɣ, and CD8/IL-10 was done. The reveal the reaction DAB+H_2O_2 and Esmerald Chromogens were used. The immunostained cells were quantified considering the pattern of staining as well as cell morphology. The cellular density (the number of cells per square millimeter) of each marker was determined by the ratio between the immunostained cells and the area of each photo. Immunohistochemical analysis showed a density (average ± standard error) of 379.1 ± 80.5 for $CD4^+$ cells and 952.5 ± 245.6 cells/mm^2 for $CD8^+$ cells, showing higher involvement of CD8 than CD4 cells. The quantitative analysis of double-stained immunohistochemistry for T lymphocyte subpopulations showed a density of 151 ± 31 for $CD4^+$/IFN-ɣ$^+$ cells, 73.1 ± 14.1 for $CD4^+$/IL-10^+ cells, 700 ± 87 for $CD8^+$/IFN-ɣ$^+$ cells and 88.8 ± 13.8 for $CD8^+$/IL-10^+ cells/mm^2. The ratio of CD4/IFN-ɣ:CD4/IL-10 was 2.1 and the ratio of CD8/IFN-ɣ:CD8/IL-10 was 7.9. The results showed that in the skin lesions of patients affected by NUCL, the *in situ* cellular immune response is marked by the predominant involvement of $CD8^+$/IFN-ɣ$^+$ cells followed by $CD4^+$/IFN-ɣ$^+$ cells ($p < 0.05$), suggestion that Tc1 and Th1 cells could play an important role in NUCL caused by *L. (L.) infantum chagasi* activating macrophages to produce toxic metabolites which help the control of tissue parasitism and the evolution of the skin lesion size.

Keywords *L.* (*L.*) *infantum chagasi;* NON-ULCERATED CUTANEOUS LEISHMANIASIS; IMMUNOHISTOCHEMISTRY; LYMPHOCYTES; CYTOKINES

Financing FAPESP #2014/50315-0, #2017/24834-9, #2018/04698-6, CAPES, CNPq e LIM50 HC-FMUSP

P2-056: PHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF EXHAUSTED T-CELL SUBSETS DURING *Leishmania mexicana* INFECTION

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Leishmania mexicana is the main species responsible for cutaneous leishmaniasis in southeastern Mexico and part of Central America. According to the clinical manifestations, it can be divided into localized cutaneous leishmaniasis and diffuse cutaneous leishmaniasis, a rare chronic variant with incomplete response to anti-leishmania treatment and frequent relapses. The functional impairment of T cells during diffuse leishmaniasis has been strongly related to the acquisition of an "exhausted" phenotype associated with PD-1 up-regulation. Heterogeneous subsets within exhausted $CD8^+$ T cells that mediate tumor and viral control after PD-1/PD-L1 blockade have been recently described, however, little is known about these subpopulations in leishmaniasis. In this study, we identified a $CXCR5^+PD\text{-}1^+$ progenitor-like and $CXCR5^-PD\text{-}1^+$ terminally exhausted cells within $CD4^+$ and $CD8^+$ compartments in draining lymph nodes (dLNs) of C57BL/6 mice infected with stationary-phase promastigotes of *L. mexicana* ($1x10^5$) in the hind footpad. Longitudinal analysis showed that $CXCR5^+$ subset was beginning to differentiate at 6 weeks post- infection and gradually increased up to 12 weeks as parasite burden was augmented in footpad lesions, while $CXCR5^-$ cells were detectable until 8 weeks post-infection, showing that persistent antigen stimulation drives the differentiation of these exhausted T cells. Notably, we found that $CXCR5^+PD\text{-}1^+$ T cells obtained from dLNs at 12 weeks of infection expressed lower levels of PD-1 and TIM-3 than their $CXCR5^-$ counterparts upon antigen-specific stimulation with promastigotes antigen (pLAg). Consistently, we observed that frequency of Ki-67 and IFN-γ expressing cells was higher in

these CXCR5^{+}PD-1^{+} progenitor- like cells after policlonal (Concanavalin A or PMA/Ionomycin) or pLAg stimulation, indicating a more terminally exhausted state in CXCR5^{-} subset. Moreover, CXCR5^{-}PD-1^{+} CD8^{+} T-cell compartment was almost negative for degranulation marker CD107a, suggesting a lower cytotoxic effect against infected cells. In summary, we have identified a novel CXCR5^{+}PD- 1^{+} progenitor-like cells that might mediate the control of *L. mexicana* infection through their proliferative potential, pro-inflammatory cytokine production and cytotoxic capacity. A better understanding of exhausted T-cell subsets during *Leishmania* infections represent a novel approach to improve and develop new therapies that afford long-term protection from chronic forms of leishmaniasis.

Keywords CUTANEOUS LEISHMANIASIS; C57BL/6; PD-1; T-CELL EXHAUSTION; PROGENITOR-LIKE SUBSET

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P2-059: THE IMPACT OF TYPE 1 INTERFERON (IFN1) SIGNALING IN GAMMA DELTA (γδ) T CELL IN *LEISHMANIA AMAZONENSIS* INFECTION

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γδ T cells are a class of lymphocytes that express the TCRγδ$^+$ being a minority fraction of circulating T lymphocytes (0.5-5%), however, they are found in large proportions in epithelial tissues such as skin, intestine and secondary lymphoid organs such as the spleen and lymph nodes (40-70%). The role in some infectious diseases is still unclear as in leishmaniasis a set of neglected diseases caused by parasites of the *Leishmania* genus that spreads mainly in tropical and subtropical areas. They are divided into cutaneous leishmaniasis (CL) or visceral leishmaniasis (VL) and VL can be fatal if left untreated. According to literature data, the role of γδ T cells in CL seems to depend on the parasite strain and animal background. Recently, our group demonstrated that Sv129 mice susceptible to *Leishmania amazonensis* infection fail to induce IFN-γ-producing CD4 and CD8 T cells and, significantly, induce the expansion of IL-17A-producing γδ T cells (IL-17A$^+$ γδ T cell).Mice were infected with 2 x 10^6 *L. amazonensis* promastigotes (MHOM/BR/75/Josefa strain) on the right hind footpad. The progression of the infection was monitored weekly by increasing the thickness of the footpad using a caliper. At the end of the experiment, popliteal lymph node cells were collected and analyzed by flow cytometry according to specific markers for each cell and cytokine. It is already known that IFNAR1 signaling down-regulates IL-17A$^+$ γδ T cell expansion during bacterial infections. We decided to evaluate the IFNAR1 signaling in *L. amazonensis*

infection. Using IFNAR1-deficient (IFNAR1 KO) mice infected with *L. amazonensis*, we observed that these animals were more susceptible to infection compared to wild-type mice (WT). IFNAR1 KO mice had a higher percentage of IL-17A^{+} γδ T cell in comparison to WT mice. We also observed that γδ T cells subdivide into γδCD3^{High} and γδCD3^{Low} and that in IFNAR1 KO mice there is a higher percentage of IL-17A^{+} γδCD3^{Low} in comparison to γδCD3^{High} and WT mice. Together, our results demonstrate that IFNAR1 signaling controls γδ T cell expansion during *L. amazonensis* infection and higher lesions are correlated to increase of IL-17A^{+} γδ T cells.

Keywords γδ T CELLS; IFNAR1; *L. amazonensis*

Financing CAPES, CNPq, FAP

P2-060: CD8⁺ T-CELLS SHOWED GREATER PROMINENCE THAN CD4⁺ T-CELLS TO ANTIMONY THERAPY IN ANY OF THE CLINICAL FORMS OF CUTANEOUS LEISHMANIASIS CAUSED BY *Leishmania (L.) amazonensis*

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In Brazil, seven *Leishmania* species and one hybrid parasite are associated with American cutaneous leishmaniasis (ACL), all occurring in the Amazon. Of these, *Leishmania (L.) amazonenis* stands out with a wide clinical-immunopathological spectrum, being responsible for three clinical forms: localized cutaneous leishmaniasis (LCL: DTH$^{\pm}$/TCD4^{+}<TCD8^{+}/Th1>Th2, good prognosis for treatment), borderline disseminated cutaneous leishmaniasis (BDCL: DTH^{-}/TCD4^{+}<TCD8^{+}/Th1≥Th2, long treatment prognosis) and anergic diffuse cutaneous leishmaniasis (ADCL: DTH^{-}/TCD4^{+}<TCD8^{+}/Th1<Th2, poor prognosis treatment), in which there is an increasing suppression of cellular immunity starting from the LCL, passing through the BDCL and ending with the ADCL. However, although a higher density of CD8^{+} T-cells than CD4^{+} T-cells density prevails along the spectrum, we decided to examine the dynamics of the both T-cell types in the three clinical forms during antimony therapy (15mgSB^{v}/kg/weight, twenty-five days each series), comparing cell density before and after the 1st and 2nd series of therapy, aiming to evaluate the potential of each T-cell type in the disease resolution. Thus, three sequential biopsies of skin lesion from one patient of each clinical form were collected, the first before and two after the 1st and 2nd series of antimony therapy. Paraffin-embedded biopsies were carried out for immunohistochemical analysis of histological

sections using monoclonal antibodies anti-CD4 (M7310) and anti-CD8 (M7103) T-cells [DAKO]. A Zeiss image analysis system was used to quantify immunostained cells [5-8 fields/histological section (400x)] and $CD4^+/CD8^+$ T-cells densities (cells/mm^2) were analyzed by Mann-Whitney test using Bio-Stat 5.0 ($P<0.05$). Results revealed a significant ($P<0.05$) increase in $CD8^+$ T-cells expression after starting antimony therapy not only in the three clinical forms, but also from the ADCL form to the LCL one [LCL: 1754, 3762, 3468; BDCL: 1875, 2948, 2234; ADCL: 925, 1743, 1426], whereas no difference was observed in $CD4^+$ T-cells expression not only in the three clinical forms, but also between these clinical forms [LCL: 1473, 1296, 1563; BDCL: 1628, 1691, 1347; ADCL: 1165, 985, 1024]. These data strongly confirm the central role of $CD8^+$ T-cells, rather than $CD4^+$ T-cells, in cellular immunity against *L. (L.) amazonensis*-infection, as well as in the resolution of the different clinical forms of the disease.

Keywords $CD8^+/CD4^+$ T-CELLS; IMMUNOHISTOCHEMICAL; AMERICAN CUTANEOUS LEISHMANIASIS; *Leishmania (l.) amazonnsis*

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P2-061: *Leishmania (V.) lainsoni* AXENIC AMASTIGOTE ANTIGEN WAS MORE REACTIVE THAN THAT OF L. (V.) braziliensis PROMASTIGOTE WITHIN THE MONTENEGRO SKIN TEST

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The laboratory diagnosis of American cutaneous leishmaniasis (ACL) still needs a tool accessible to the epidemiological reality of the disease in Brazil. Montenegro skin test (MST) currently uses crude antigen from promastigote form of *Leishmania* sp., however, it is well-known that the amastigote form of the parasite that modulates cellular and humoral immune responses against to infection. Thus, considering that *Leishmania (V.) lainsoni* is a strongly cellular immunity-inducing species and easy to grow in the laboratory, we decided to evaluate the reactivity to crude, stage-specific, *L. (V.) lainsoni* axenic amastigote antigen in the ACL diagnosis by the MST. In this order, thirty-one (31) patients with ACL clinical, parasitological (conventional and/or molecular) and/or serological diagnosis were selected, twenty-four (24) with localized cutaneous leishmaniasis (LCL) and seven (7) with mucosal leishmaniasis (ML), treated at the Evandro Chagas Institute' leishmaniasis clinic, Ananindeua, Pará State, Brazil. To evaluate the MST reactivity with *L. (V.) lainsoni* axenic amastigote antigen (173,3 µg/mL= 17,3 ng/mL/dose protein), a crude *L. (V) braziliensis* promastigote antigen (241,2 µg/mL= 24,1 ng/mL/dose protein) was used simultaneously on the opposite forearm of each patient. The comparison of the MST reactivity between AMA [*L. (V.) lainsoni* amastigote] and PRO [*L. (V.) braziliensis* promastigote] antigens was performed by the paired t-test. The overall value (mean ± standard deviation) of the MST reactivity to the thirty-

one patients with AMA antigen (21.0 mm ± 8.5) was higher (*P*< 0.001) than that with PRO antigen (11.8 mm ± 5.4). When the MST reactivity was evaluated by the ACL clinical form, it was observed that AMA antigen values for LCL (20.8 mm ± 8.5) and ML (21.4 mm ± 8.6) were also higher (*P*< 0.001) than those for LCL (11.6 mm ± 5.3) and ML (12.6 mm ± 5.4) with PRO antigen. In addition, it is significant to inform that, of the thirty-one patients examined, in only two with LCL form the causal agent was *L. (L.) amazonensis*, in which the MST reactivity was only confirmed with *L. (V.) lainsoni* AMA antigen. These results clearly evidenced a greater ability (almost twice) of the *L. (V.) lainsoni* AMA antigen to promote a specific MST reactivity than that of the *L. (V.) braziliensis* PRO antigen, thus revealing to be a strong candidate to compose, in the near future, the MST antigen for the ACL laboratory diagnosis.

Keywords *Leishmania (v.) lainsoni*; AXENIC AMASTIGOTE ANTIGEN; MONTENEGRO SKIN TEST; AMERICAN CUTANEOUS LEISHMANIASIS

Financing Evandro Chagas Institute/SVS,MS,Tropical Medicine Nucleus, Federal University of Pará, Brazil

P2-063: ROLE OF TLR9 IN THE EXPRESSION OF CD200 MODULATING *LEISHMANIA AMAZONENSIS* VIRULENCE *IN VIVO*.

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Leishmaniasis is a complex of devastating diseases affecting different tropical and subtropical areas by virulent protozoan species named *Leishmania*. The virulence of these parasites depends on the capacity of infective forms named amastigotes to modulate and inhibit the host immune response. Amastigotes of *Leishmania amazonensis* induce the expression of the host ligand CD200, an immunomodulatory glycoprotein, which in contact with its receptor (CD200R) inhibits iNOS/NO signaling pathways favoring intracellular survival. CD200 induction depends on the activation of TLR9/MyD88/TRIF signaling pathways by DNA-containing extracellular vesicles released by intracellular amastigotes. Here we investigate the infection process and analyze the macrophage population ($CD45^+CD11b^+F4/80^+$) recruited at the lesion in the footpads of WT and $TLR9^{-/-}$ mice by flow cytometry infected with *L. amazonensis*. As early as the third week of infection, infected $TLR9^{-/-}$ mice developed much smaller lesions, containing a reduced number of parasites recovered at the end of the experiment. As expected, WT mice showed rapid growing and non-healing lesions typical of *L. amazonensis* infection that had an increased parasite load in the infected tissue, relative to mild infection observed in $TLR9^{-/-}$ mice. When recovered from the lesions, double positive CD45 and CD200 expressing cells ($CD45^+CD200^+$) were significantly higher in WT than $TLR9^{-/-}$ mice. More importantly, a marked difference was observed in the absolute number of macrophages ($CD45^+CD11b^+F4/80^+$ cells). Finally, WT mice contained 3 folds more macrophages than $TLR9^{-/-}$ mice, and those cells expressed significantly more CD200 than the normalized number of macrophages from $TLR9^{-/-}$ mice. We showed that the impairment of CD200

expression reduces macrophage recruitment in TLR9$^{-/-}$ mice lesions. Further studies will describe the kinetic of CD200 induction and the other cells involved in controlling immune response modulated by *Leishmania* parasites.

Keywords AMASTIGOTES; CD200; *Leishmania amazonensis*; MACROPHAGES; TLR9

Financing FAPESP; CNPq; CAPES

P2-064: FURTHER EVIDENCE ON INTRIGUING *Leishmania (L.) amazonensis* INTERACTION WITH T-CELL IMMUNE RESPONSE IN AMERICAN CUTANEOUS LEISHMANIASIS

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American cutaneous leishmaniasis (ACL) is a parasitic protozoan disease caused by *Leishmania* species belonging to the subgenera *Viannia*, *Leishmania* and *Mundinia* widely distributed in Latin America. In Brazil, seven *Leishmania* species have been implicated with ACL, of which *L. (L.) amazonensis* may give rise to a wide clinical-immunopathological spectrum ranging from localized cutaneous leishmaniasis (LCL: $DTH^{\pm}/TCD4^{+}<TCD8^{+}/Th1>Th2$), which usually responds well to antimony therapy, toward to borderline disseminated cutaneous leishmaniasis (BDCL: $DTH^{-}/TCD4^{+}<TCD8^{+}/Th1\geq Th2$), that requires the double of LCL-therapy antimony regime to heal and, anergic diffuse cutaneous leishmaniasis (ADCL: $DTH^{-}/TCD4^{+}<TCD8^{+}/Th1<Th2$), which is marked by resistance to any type of chemotherapy. In this way, it seems clear that human *L. (L.) amazonensis*-infection is modulated by different degrees of specific T-cell immune suppression starting from the LCL form ($DTH^{\pm}/Th1>Th2$), passing through the BDCL ($DTH^{-}/Th1\geq Th2$) and going to the ADCL ($DTH^{-}/Th1<Th2$). The main subject of this work concerns the clinical-immunological evolution of three LCL cases due to *L. (L.) amazonensis* treated at the leishmaniasis laboratory "Ralph Lainson", in the “Evandro Chagas Institute”, Pará State, Brazil, which, even after establishing the clinical cure of the disease, did not convert the DTH response known to be one of the most effective mechanisms of the T-cell immune response. All three cases had a single infiltrated cutaneous lesion, evolving from two to

four months. The laboratory diagnosis was confirmed by parasite search in skin lesions, with isolation and specific characterization of the parasite by molecular biology (PCR). One of these cases refused to undergo antimony therapy (15mgSB^{v}/kg/weight, twenty-five days each series/two series with interval of seven to ten days), and the other two successfully underwent therapy. The patient who refused therapy was re-examined one year after the first evaluation [the lesion had spontaneously healed about six months ago] and the other two were re-examined ten days after the second course of therapy, when they were challenged again by DTH, but none of them converted the reactivity to DTH. The present results, although have been based on the observation of only three LCL cases by *L. (L.) amazonensis* [in view of low frequency (2-4%) of LCL by *L. (L.) amazonensis* as well as the difficulty to perform DTH in previously cured patients], reveal how intriguing and complex are *L. (L.) amazonensis*' escape mechanisms from the T-cell immune response in ACL, even though the patients have managed to cure the disease.

Keywords AMERICAN CUTANEOUS LEISHMANIASIS, *Leishmania (l.) amazonensis*, T-CELL IMMUNE RESPONSE

Financing Evandro Chagas Institute/SVS,MS,Tropical Medicine Nucleus, Federal University of Pará, Brazil

P2-065: ACTIVATION OF NLRP3 AND AIM2 INFLAMMASOMES IN AMERICAN CUTANEOUS LEISHMANIASIS CAUSED BY *LEISHMANIA (L.) AMAZONENSIS* AND *L. (VIANNIA) BRAZILIENSIS*

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American cutaneous leishmaniasis (ACL) has a wide spectrum of clinical and immunopathological manifestations resulting from the interaction between different species of *Leishmania* and the hosts immune response mechanisms. At the center of the spectrum, where there is a balance of immune response, are the localized cutaneous leishmaniasis (LCL); in the hyporeactivity and hyperreactivity poles are, respectively, anergic diffuse cutaneous leishmaniasis (DCL), caused by *L. (L.) amazonensis*, and mucosal leishmaniasis (ML), caused by *L. (V.) braziliensis*. Recent studies have demonstrated critical roles for cytoplasmic sensors and inflammasomes in *Leishmania* spp. However, to date, the activation of these inflammasomes in the clinical-immunopathological spectrum of ACL caused by *L. (L.) amazonensis* and *L. (V.) braziliensis* has not been evaluated. The aim of this work was to determine the participation of AIM2 and NLRP3 inflammasomes, as well as the indirect components (IL-1β, IL-18 and caspase-1), in patients with different clinical forms of ACL caused by *L. (L.) amazonensis* and *L. (V.) braziliensis* through of immunohistochemistry

reactions. The density of immunostained cells was determined using Image Analysis System using Axio Vision 4.8.2 software (Zeiss). *In situ* expression of IL-18, IL-1β, caspase-1, NLRP3 and AIM2 was positive in all lesions analyzed. IL-1β and caspase-1 expression was higher in the more severe clinical forms of the disease (IL-1β: ADCL [1135.35 cells/mm^2]; MCL [1159.72 cells/mm^2]) (Caspase-1: ADCL [875.80 cells/mm^2); ML [768.06 cells/mm^2). The density of IL-18+ cells was significantly higher in the clinical form ADCL [816.65 cells/mm^2] when compared to LCM [334.79 cells/mm^2]. AIM2 expression was higher in both the most severe and polar clinical forms of ATL [ADCL: 941.42 cells/mm^2] [MCL: 869.97 cells/mm^2]. However, NLRP3 inflammasome expression was higher in MCL [661.11 cells/mm^2] when compared to ADCL [332.04 cells/mm^2]. Thus, our data show that both inflammasomes participate in the immune response of patients in the clinical and immunopathological spectrum of ACL. In ADCL, a higher density of AIM2+ cells was observed when compared to NLRP3+, suggesting a greater activation of AIM2 in this hyporeactive form. In the MCL hyperreactive form, a high density of both AIM2+ and NLRP3+ cells was demonstrated, suggesting that these inflammasomes may play a role in parasitism control, but also contribute to disease severity when their expression is increased in the lesion. The expression of IL-1β and caspase-1 was higher in the most severe forms of the disease, suggesting an anti-inflammatory role in ADCL and a pro-inflammatory role in MCL. Studies with *L. (L.) amazonensis* showed that IL-18 contributes to a host susceptibility profile, corroborating our findings. The low expression of IL-18 in LCL caused by *L. (V.) braziliensis* corroborates previous studies of our group with *Leishmania (V.) panamensis*. Our findings showed the participation of AIM2 and NLRP3 inflammasomes and their indirect components (IL-1β, IL-18 and caspase-1) in the spectrum of ACL caused by *L. (L.) amazonensis* and *L. (V.) braziliensis*, suggesting that the activation of these inflammasomes is possibly associated with the severity of the polar forms of the ACL spectrum (ADCL and ML).

Keywords AMERICAN CUTANEOUS LEISHMANIASIS; INFLAMMASOMES; NLRP3; AIM2

Financing Grant # 2014/50315-0, São Paulo Research Foundation (FAPESP), CAPES; LIM50 HC-FMUSP; Instituto Evandro Chagas (SVS/MS); UFPA-Pará, Brasil

P2-066: INFLAMMASOME GENE EXPRESSION IN THE ANERGIC (DIFFUSE) AND HIPERERGIC (MUCOSAL) FORMS OF AMERICAN CUTANEOUS LEISHMANIASIS

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American cutaneous leishmaniasis (ACL) is a neglected infectious disease caused by different *Leishmania* species widely distributed throughout Latin America. In Brazil, *L. (L.) amazonensis*[*La*] and *L. (Viannia) braziliensis[Lb]* have highest potential to cause the most severe clinical forms ACL, mucosal leishmaniasis (ML) and anergic diffuse cutaneous leishmaniasis (ADCL),respectively. Recent studies have shown that innate immune response mediates the assembly of inflammasomes, which are multiprotein intracellular receptors platforms that activates a caspase, IL-1β and IL-18 production, and the cell death by pyroptosis. Thus, although these studies have demonstrated a significant role of inflammasomes in the pathogenesis of ACL, this represents the first study concerning the inflammasome gene expression on the polar forms of the disease, i.e., the anergic (ADCL) and hyperergic (ML) forms. To better understand the transcriptional status of pathways and genes related to inflammasomes, next-generation RNA sequencing-(RNAseq) was accomplished with 10 skin biopsies of patients from the Brazilian Amazon with ADCL/*La* (n=5) and ML/*Lb* (n=5). The

mRNA expression of both groups was compared to that found in 6 healthy skin samples(control). The enrichment analysis of biological pathways(GSEA and Reactome databases) was filtered by NES values>1 or <-1 and $P_{adjusted}$<0.05. To determine differentially expressed genes(DEG) the edgeR tool was used based on log2 fold-change>1 or <-1, and $P_{adjusted}$ <0.05, revealing up- and down-regulated genes. Our results showed that the pathways "inflammasomes" and "nucleotide-binding domain, leucine rich repeat containing receptor(NLR) signaling pathways" were significantly upregulated in both the anergic ADCL form(NES=1.60, $P_{adjusted}$≤0.001; NES=1.60 $P_{adjusted}$ ≤0.001) and ML hyperergic form**(**NES=1.58, $P_{adjusted}$≤0.05; NES=1.65, $P_{adjusted}$≤ 0.001). A set of genes that compound the pathways were overexpressed (Padj≤0.01) in ADCL, but in higher extent in ML: AIM2(ADCL log_2FC=3.63; ML log_2FC=6.25), CASP5(ADCL log_2FC=1.36; ML log_2FC=3.29), IL1B(ADCL log_2FC=1.59; ML log_2FC=5.53), TNF(ADCL log_2FC=3.32; ML log_2FC= 4.57) and CASP1(ADCL log_2FC=0.78; ML log_2FC=2.06). AIM2 is a key sensor in the inflammatory process capable of recognizing the DNA of pathogens, leading to assembly of AIM2 inflammasome, which has already been associated with disease severity in ML. CASP1 and CASP5 are members of the caspase family, whose sequential activation plays a central role in the proinflammatory programmed cell death, the pyroptosis, characterized by the secretion of IL-1β. This cytokine has been associated with the severity of the disease in patients with ADCL. TNF is related to tissue necrosis process, characteristic present in patients with ML. An important finding was the identification of genes that were exclusively upregulated in ML, such as the TXN and CASP4, which contribute to the parasite control inside the macrophages, and NFKB1, which encodes the NF-κB, important nuclear factor the primes the inflammasome activation by inducing proinflammatory genes. In summary, while inflammasome-induced inflammation contributes to pathology, it also accounts for limiting parasite replication Despite both inflammasomes were activated in the polar and severe forms of ACL in our study, genes related to necrosis, inflammation and pyroptosis were higher expressed in ML compared to ADCL form, suggesting the inflammasome involvement not only in parasite restrain, but in the uncontrolled tissue inflammation and destruction that are seen in ML cases.

Keywords AMERICAN CUTANEOUS LEISHMANIASIS; INFLAMMASOME; NLRP3; AIM2; SKIN TRANSCRIPTOME GENE EXPRESSION

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P2-068: ROLE OF MACROPHAGE SPHINGOSINE KINASE ACTIVITY DURING THE MATURATION OF PHAGOSOMES HARBOURING *Leishmania mexicana* PROMASTIGOTES

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Leishmania is the name of a genus of vector borne parasitic protozoans that cause a spectrum of diseases collectively known as leishmaniasis. During their life cycle, Leishmania parasites alternate between an extracellular flagellated form (promastigote) located within the vector intestine, and an intracellular non-motile form (amastigote) that parasitizes different mammalian phagocytic cells, particularly macrophages, within which they survive and multiply, at the interior of parasitophorous vacuoles. After the internalization of most microorganisms, macrophage phagosomes undergo a process called maturation, which involves the acquisition of microbicidal properties through: pH reduction, generation of reactive oxygen species (ROS), and production of nitric oxide (NO). Leishmania promastigotes prevent their elimination by interfering with this process. Previous works from our laboratory demonstrated that *Leishmania mexicana* promastigotes restrict the translocation of the macrophage enzyme sphingosine kinase 2 towards the periphery of nascent phagosomes during their internalization. Sphingosine kinases (SK1 and SK2) catalyze the phosphorylation of sphingosine, generating sphingosine 1-phosphate (S1P), a powerful messenger involved in the regulation of numerous cellular processes, among them, Ca+2 release from internal reservoirs, a necessary condition for the fusion of lysosomes to phagosomes. In this work we set up to determine the role of the macrophage SK activity during the maturation of phagosomes harbouring *L. mexicana* promastigotes and to evaluate whether the parasite inhibits this activity as a survival strategy. For this, murine

macrophages (J774) were cultured with alive or paraformaldehyde (PFA)-fixed *L. mexicana* promastigotes in the presence of DL-threo-dihydrosphingosine (DMS, a SKs activity inhibitor) or S1P (SKs product), in order to determine if the inhibition of SK activity interfered with the phagosome maturation after internalization of PFA-fixed promastigotes; and to establish whether the addition of S1P allowed to restore the ability of macrophages loaded with live parasites to carry out this function. This was performed by: colocalization analysis of fluorophores used to stain parasites (CFSE) and macrophage lysosomes (lysotracker red) by confocal microscopy; determination of ROS production by macrophages, by NBT reduction assay; and determination of NO production, using the Griess assay. We demonstrated the participation of SK activity in the induction of phagosome maturation during the internalization of fixed *L. mexicana* promastigotes, as the acidification of this compartment, the generation of ROS and the production of NO were reversed in presence of the SK enzyme inhibitor DMS. On the other hand, it was established that live promastigotes interfere with the maturation of the phagosomes by inhibiting the SK activity of the host cell, since addition of external S1P restored the ability of the macrophage to perform this process.

Keywords LEISHMANIA; MACROPHAGE; PHAGOSOME; SPHINGOSINE KINASE; SPHINGOSINE 1-PHOSPHATE.

P2-069: PHENOTYPIC CHARACTERIZATION OF MACROPHAGES INFECTED WITH *Leishmania (L.) amazonensis*

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Leishmaniasis is a neglected tropical disease caused by parasites of the genus *Leishmania*, with *Leishmania (Leishmania) amazonensis* as species mainly associated with cutaneous and diffuse cutaneous leishmaniasis cases. Several studies have shown that infective forms of *L. (L.) amazonensis* modulate immune-regulatory molecules, such as CD200 and its receptor CD200R, inhibitory immune complex participating in the control of the immune system. CD200R is modulated and CD200 induced during *L. (L.) amazonensis* amastigotes infection, resulting in a self-inhibitory mechanism that impairs the iNOS/NO mechanism, favoring parasite resistance and proliferation. Thus, phenotypic characterization of $CD200^{+}CD200R^{+}$ macrophages infected with *Leishmania* is of vital interest in the area of parasite immunobiology. The aim is to characterize phenotypically *L. (L.) amazonensis*-infected macrophages using specific cell surface markers, including CD200/CD200R. Phenotypic characterization was performed in RAW 264.7 cells infected with axenic amastigotes of *L. (L.) amazonensis*. As controls, we used cells stimulated with IFNγ/LPS and uninfected cells. The infection was carried out for 1 hour, and the cells were processed, labeled with monoclonal antibodies for flow cytometry analysis. We obtained a total macrophage population defined by the conserved markers $CD45^{+}$, $F4/80^{+}$, $CD11b^{+}$, $CD11c^{+}$. According to the mean fluorescence intensity (MFI), markers such as CD80 (M1 population) and FcεRI (M2 population) had a constant expression profile between infected and uninfected cells. CD200R did not show a significant difference in expression; however, CD200 showed a suggestive increase of its expression in infected cells (* p-value: 0.0107) compared to non-infected cells. In

addition to this, the MFI normalization (nMFI) confirmed the results regarding CD200 increasing levels when cells are infected with *L. (L.) amazonensis*. These results are a preliminary approach to the characterization of infected macrophages at the early stages of the host-pathogen interaction, when CD200 is modulated. Future experiments will be performed evaluating other differential markers in different periods during the course of the *Leishmania*-macrophage interaction; verify the intracellular number of parasites in the cells (essential for the induction of CD200) by immunofluorescence, and CD200/CD200R expression by western blot, to verify its presence in the different conditions of this cell population.

Keywords AMASTIGOTES; CD200; CD200R; *Leishmania (L.) amazonensis*; MACROPHAGES; RAW 264.7

Financing FAPESP; CNPq; CAPES

P3-058: EXTRACELLULAR VESICLES OF *LEISHMANIA AMAZONENSIS*: ACTIVATION OF MURINE MACROPHAGES AND IMMUNOGENICITY

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Leishmaniasis is a group of diseases caused by protozoa of the genus *Leishmania*, which is transmitted to man and other mammals through the bite of selected sand flies. There are two clinical forms of the disease: visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL). They belong to the neglected diseases group, which is responsible for millions of people's death and disability each year, highlighting the need for attention. The role of extracellular vesicles (EVs) from *Leishmania* has been widely studied in recent years. Findings have pointed to an immunomodulatory function favorable to infection. Thus, this work aimed to analyze the immunogenicity of EV proteins from promastigote forms of three *L. amazonensis* strains and investigate the effect of their EVs on the activation of murine macrophages. This study was organized in four phases: (i) EVs purification from axenic cultures of *L. amazonensis* promastigotes, strains M2269, BA125, and BA336; (ii) the effect of VEs on MØ activity was analyzed by quantifying urea through colorimetric assays, and IL-1β expression and of class I (MHC I) and class II (MHC II) molecules of the major histocompatibility complex by flow cytometry; and (iii) EVs proteins' profile

was investigated by electrophoresis in polyacrylamide gel in the presence of SDS (SDS-PAGE), two-dimensional electrophoresis (2-DE) and (iv) EVs proteins' immunoreactivity was investigated by Western-blot assay using serum from mice infected with *L. amazonensis* and serum from humans with American Cutaneous Leishmaniasis (ACL). Our results showed that MØ stimulated with EVs produced more urea than resting MØ and exhibited a significant reduction in IL-1β generation. Moreover, *L. amazonensis* EVs do not interfere with the expression of MHCI and MHCII. SDS-PAGE showed a similar protein profile of EVs purified from *L. amazonensis* strains BA125 and BA336, but different from strain M2269. However, EVs proteins are recognized by serum of BALB/c mice previously infected with *L. amazonensis* and by humans with ACL. Therefore, EVs share antigens with the parasite, such as the surface gp63 and the cytoplasmic HSP70, do not promote pro-inflammatory environments and seem to avoid antigenic presentation, minimizing the activity of cellular immunity and favoring infection and parasite survival. Further studies are needed to clarify the effects of EVs on ACL, including the identification of other proteins which can play an important role in the immune microenvironment.

Keywords *Leishmania amazonensis*; EXTRACELLULAR VESICLES; IMMUNOGENICITY

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P3-059: *Leishmania mexicana* PROMOTES PAIN-REDUCING METABOLIC REPROGRAMMING IN CUTANEOUS LESIONS

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Cutaneous leishmaniasis (CL) is characterized by extensive skin lesions, which are often painless, indicating that *Leishmania* infection may trigger nociceptive activities in the infected area. Yet the molecular mechanisms responsible for this clinical phenomenon have not been identified. Through an unbiased metabolomic analysis by mass spectrometry, we found elevated levels of caffeine metabolites at the lesion site during chronic infection in murine models with *Leishmania* (*L.*) *mexicana,* as well as *in vitro* in infected macrophages, compared to their non-infected counterparts. Caffeine metabolites have known anti-inflammatory and analgesic properties by acting through adenosine receptors and inhibiting transient receptor potential vanilloid 1 (TRPV1) channels. Additionally, analgesic effects are also mediated by purine metabolites that promote IL-10 production, by dampening inflammation and by directly reversing sensory neuron hyperexcitability. Furthermore, we found arachidonic acid metabolism enriched in the ear lesions, compared to the non-infected controls. Arachidonic acid is a metabolite of anandamide (AEA) and 2-arachidonoylglycerol (2-AG). These endocannabinoids act on cannabinoid receptors 1 and 2 and TRPV1 channels to exert anti-inflammatory and analgesic effects. We validated our mass spectrometry results by measuring the expression of enzymes in the arachidonic acid pathway via RT-PCR. We found significantly higher expression of N-acylphosphatidylethanolamine-phospholipase D (NAPE-PLD), a key enzyme involved in AEA biosynthesis,

and significantly lower expression of fatty acid amide hydrolase (FAAH), which mediates both AEA and 2-AG degradation. Our study provides the first evidence of metabolic pathways upregulated during *L. mexicana* infection that may mediate anti-nociceptive effects experienced by CL patients and identifies macrophages as a source of these metabolites.

Keywords ANALGESIA; CUTANEOUS LEISHMANIASIS; METABOLIC REPROGRAMMING; METABOLOMICS

P3-060: EVALUATION OF THE INTERACTION BETWEEN *LEISHMANIA* SPECIES DURING IN VITRO CULTIVATION

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Oswaldo Cruz Foundation

The maintenance of the microbial community depends on, among other things, the interaction between microorganisms that compose that community. This feature has been poorly explored in the protozoan of the genus *Leishmania*. The interactions and diversity within the population of cells can impact the biology of the parasite and thus the interplay with vertebrate and invertebrate hosts. In the field there are evidences of polyclonal, multiple *Leishmania* strain or species in the course of infection. These infections may be associated with alterations in the clinical outcome, response to treatment, and transmissibility. It has been reported by *in vitro* assays that species or strains influence each other's development. Such an effect depends on physical contact between the parasites, and/or on secreted factors. *Leishmania* parasites secrete *in vitro* and *in vivo* proteins and vesicles that influence the maintenance of the microbial community, contributing positively or negatively to the establishment of the infection. These considerations led us to question whether there is an inhibitory (fitness loss) or stimulatory (fitness gain) effect during the interaction between *Leishmania* species in vitro. We assessed the kinetic growth of *Leishmania (Viannia) naiffi* (IOC/L3310) and *L. (V.) braziliensis* (IOC/L 3356) up to the fifth passage in single and co-cultivation in both Schneider and M199 culture medium, FCS 20% (strains obtained from the Leishmania Collection from FIOCRUZ - CLIOC). The parasites were counted daily for 336 hours (or 14 days). DNA was isolated in each timepoint and quantified by qPCR to determine the parasite composition in culture. The effect of heterologous culture supernatant containing secreted molecules on parasite growth and cell viability was measured by a colorimetric assay based on resazurin. Results show growth curves of the single *L. naiffi* culture and the

L. naiffi / *L. braziliensis* co-cultivation exhibit similar profiles, and both depicted higher density when compared to single *L braziliensis* culture. qPCR confirmed the higher density of *L. naiffi* along the co-culture. *L. naiffi* cultivated with the heterologous supernatant obtained from *L. braziliensis* cultures presented higher viability and density of cells than the control at 24 hours. Conversely, *L. braziliensis* cultivated with the heterologous supernatant obtained from *L naiffi* cultures did not differ from control. However, when cultivated with its own supernatant, *L. braziliensis* also presented greater viability at 24 hours. Our results suggest there are no negative effects on parasite growth during the *in vitro* interaction between both species. Instead, viability assay indicates *L. braziliensis* secreted factors benefit both, *L naiffi* and the *L. braziliensis* itself when added to the culture medium. Further qPCR data of co-culture will be added to the cell viability assays and parasite count results allowing a deeper understanding of the interactions effects.

Keywords *Leishmania* INTERACTIONS; CO-CULTURE; CULTURE SUPERNATANT; QUORUM SENSING

P3-061: ASSOCIATION OF TESTOSTERONE AND DIHYDROTESTOSTERONE WITH THE SEVERITY OF VISCERAL LEISHMANIASIS: A LONGITUDINAL STUDY

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Despite the reduction in the global estimates of mortality from visceral leishmaniasis (VL), as well as the structuring of health programs and policies, controlling the disease is still a challenge in Brazil, which is characterized as an endemic region, with high prevalence and more cases in males. In this perspective, extensive discussions have been developed to understand the significant increase in parasitaemia in men, specifically just after adolescence, as well as to establish the influence of sex hormones such as testosterone and Dihydrotestosterone (DHT) on the synthesis of the immune response, the susceptibility and burden of disease, the clinical spectrum and the potential letality. Considering that sex hormones may have immunosuppressive potential or induce pro-inflammatory defense mechanisms, this study aimed to verify the association of serum levels of total testosterone and DHT with the clinical manifestations and severity of VL. This is a retrospective longitudinal research, conducted in a reference institution for the treatment of infectious and parasitic diseases in Teresina, Piauí, Brazil. A total of 134 men participated, where the sample composition involved the formation of two groups: 113 patients admitted in the period 2008 and 2020 with VL diagnosis, and 21 healthy. Among the patients, 25 participants were evaluated before the beginning of treatment and thirty days after. The primary endpoint considered was the serum concentration

of total testosterone and DHT, as well as clinical manifestations and the probability of death, calculated using the Kala-Cal® software. The analysis was expressed by descriptive and inferential measures, using the Kolmogorov Smirnov test, Student's T test and Bonferroni correction to measure the relationship between variables. The study was approved by the Research Ethics Committee of the Federal University of Piauí, under favorable opinion number 3.152.312. The results showed homogeneity in age distribution among the groups studied, with an age range of 13 to 80 years. The manifestations involved a broad clinical spectrum, characterized by fever (88.5%), splenomegaly (87.3%), weight loss (73.5%), fatigue (73.5%), hepatomegaly (65.7%), and skin pallor (61.1%). The fatal outcome was observed in 6.2% of the mostly elderly participants. The sick group had lower mean total testosterone concentration (502.2 ng/dL, $p<0.001$) and higher DHT levels (709.6 pg/mL, $p=0.022$) when compared to the healthy group. DHT concentrations were lower in patients who had vomiting, diarrhea, dyspnea, and gum bleeding, and were also associated with a reduced likelihood of death ($p<0.001$). Therefore, it is suggested that the high level of DHT may be related to hypoalbuminemia, a typical condition of the disease, leading to higher concentration of free testosterone and DHT synthesis. Thus, DHT may help explain some phenomena observed in VL, with a probable action in attenuating the symptomatology and severity of the disease in men. Thus, it is considered that the immunomodulation of DHT may determine the immune response in VL, making men more susceptible to acquire infection by *Leishmania spp*, explaining the bias of higher incidence of VL in adolescent and adult males.

Keywords VISCERAL LEISHMANIASIS; *Leishmania infantum*; TESTOSTERONE; DIHYDROTESTOSTERONE; CLINICAL STATUS

P3-062: TISSUE TROPISM OF TWO VARIANTS OF *TRYPANOSOMA CRUZI* IN ZEBRAFISH MODEL

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Trypanosoma cruzi is the agent of Chagas disease, which could have a variable tissue tropism according to its genotypic variant, known as Discrete Typing Units (DTUs). Seven DTUs have been described, from TcI to TcVI and TcBat. During chronic human infection, the parasite can be found mainly in heart tissue and less frequently in digestive system organs. The tissue location depends on the parasite membrane adherence and cellular invasion to the mammalian host cells. Currently, *in vivo* models for *T. cruzi* have a limited capacity to directly observe live parasites in circulation and organs, which makes it difficult to study parasite-host behavior. Previous studies in zebrafish models evaluated the motility of *T. cruzi* within zebrafish larvae. The goal of this study was to assess the adherence of TcI and TcII trypomastigotes (most common DTUs) to tissues in the zebrafish larvae model. Trypomastigotes from TcI strain DA and TcII strain Y were labeled with fluorescent dyes, either CFSE (green) or FarRed (red), and then injected into zebrafish larvae 48 to 72 hours post-fertilization. Videos of the entire process were acquired in a stereomicroscope, and afterward by light-sheet fluorescence microscopy (LSFM), in order to determine the exact position of the parasites inside the zebrafish larvae. Those confirmed injected larvae underwent trypsin digestion, and parasites were detected and quantified by flow cytometry. Prior results were confirmed, and it was possible to observe *T. cruzi* TcI trypomastigotes adhered to the

atrioventricular valve, atrium, blood vessels and yolk sac wall. Upon injection with DTU TcII, parasites were adhered to cardiac tissue, mainly to the atrium. Unlike TcI, more parasites were found attached to different cardiac locations when using TcII. Also, TcII migration was seen from the duct of Cuvier to the yolk sac extension, the future digestive tract of the zebrafish. When TcI and TcII were simultaneously injected into the same larvae, they both attached to the zebrafish heart. Finally, after enzymatic digestion, it was found that parasite was detected in 27% of injected larvae. Both parasites (DTUs TcI and TcII) were found attached to the heart of larvae *in vivo*. In previous studies in mouse models, tissue tropism was associated with host genetic factors, however, here it was found that both TcI and TcII can adhere to the heart in this zebrafish model. Another tissue tropism (probably digestive tract) was detected in larvae injected with TcII. Finally, LSFM is a high-resolution and useful technique to observe living organisms and their interaction non-invasively.

Keywords CHAGAS DISEASE; PARASITIC DISEASE; INTRAVITAL MICROSCOPY; ZEBRAFISH; TROPISM

P3-063: THE IMPORTANCE OF SIALIC ACIDS IN *Leishmania* (*L.*) *amazonensis* AND *Leishmania* (*L.*) *infantum chagasi* INFECTION

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Leishmania sp. are etiological agents of leishmaniasis, one of the most important parasitic diseases in the world. *Leishmania* promastigotes are covered by a dense glycocalyx composed by many glycoconjugates, which play an important role in *Leishmania* infectivity and survival. Sialic acids (Sias) are nine-carbon atoms sugars usually present as terminal residues of glycoproteins and glycolipids on the cell surface or secreted. The role of Sias in infections by protozoa such as *Trypanosoma cruzi* and *Leishmania donovani* was demonstrated in previous studies. The interaction between *Leishmania* (*L.*) *donovani* Sias and macrophage receptor Siglec-1 (Sialic acid-binding immunoglobulin-type lectins) contributes to the parasite's entry into the host cell. Due to the divergences among *Leishmania* species, the aim of this work was to evaluate the importance of Sias-Siglec-1 interaction in two endemic species in Brazil: *L.* (*L.*) *amazonensis* and *L.* (*L.*) *infantum chagasi*. For that, we treated parasites with sialidase, removing part of *Leishmania* Sias. *In vitro* infection assays using murine bone marrow-derived macrophages (BMDM) and cells of human lineage THP-1 showed that reduction of Sias decreased infection. These results were observed for both species including two different strains of *L.* (*L.*) *infantum chagasi*, MHOM/BR/1972/LD and MHOM/BR/2005/NLC, with the greatest impact of sialidase treatment for MHOM/BR/2005/NLC strain. We then analyzed Siglec-1 abundance in murine macrophages (BMDM) and human

differentiated THP1 cells by flow cytometry. A low labeling was observed in BMDM, while more than 50% of THP1 cells expressed Siglec-1. Blocking of Siglec-1 had no impact in BMDM or THP1 cells infection. Experiments for Siglec-1 silencing in THP1 are in course at this moment. Until now, our results imply that sialic acid is important for *L.* (*L.*) *amazonensis and L.* (*L.*) *i. chagasi* infection and that the impact of sialidase treatment may vary between strains of the same species.

Keywords *Leishmania*; SIALIC ACID; MACROPHAGE INFECTION

Financing CNPq, CAPES, FAPESP

P3-064: THE ROLE OF DIFFERENT *LEISHMANIA BRAZILIENSIS* ISOLATES ON THE PATHOGENESIS OF DISSEMINATED LEISHMANIASIS

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Disseminated Leishmaniasis (DL) is an emerging and severe form of *Leishmania (Viannia) braziliensis* infection defined by the presence of 10 and up to more than 1,000 skin lesions and a high rate of mucosal involvement. DL has been associated with high rates of failure to antimony therapy. Often confused with diffuse cutaneous leishmaniasis (DCL), DL is considered clinically, immunologically and histopathologically distinct from DCL. While DCL patient's exhibit poor lymphocyte proliferation and absent or low production of IFN-γ upon exposure to soluble leishmania antigen (SLA), an impaired Th1 immune response has not been documented in DL. The mechanisms underlying parasite dissemination remain unknown. Parasite factors are also known to influence the pathogenesis of leishmaniasis. As *L. (V.) braziliensis* is polymorphic, dissimilarity among strains has been associated with different clinical forms of disease, as well as failure to antimonial therapy. The participation of monocytes is also notable in host inflammatory response against CL, as the enhancement of intermediate monocytes provides an important source of TNF, a cytokine associated with tissue damage in tegumentary leishmaniasis. The Toll-like receptor (TLR) signaling pathway is a primary defense mechanism against infectious agents. The elevated expression of TLR2 and TLR4 by intermediate

monocytes from *L.(V.) braziliensis*-infected CL patients in comparison to healthy subjects has been associated with TNF production. The present work compared the function of monocytes obtained from cutaneous leishmaniasis (CL) and DL patients in response to infection with isolates of *L. braziliensis* pertaining to both of these two clinical forms of disease. Mononuclear cells obtained from DL and CL patients were infected with different *L. braziliensis* isolates. Numbers of infected cells and parasite load were determined by microscopic evaluation of 100 monocytes following Romanowsky staining of cytocentrifuge preparations. Respiratory burst, TLR2 and TLR4 expression and cytokine production were evaluated by cytometry. DL isolates infected more monocytes, induced greater respiratory burst, higher expression of TLR2 and TLR4 and more cytokine production compared to isolates from CL patients regardless of the origin of monocytes used (DL or CL). However, greater parasite multiplication and higher TLR expression were seen in monocytes from DL patients compared to CL following infection with DL isolates. Our results indicate the participation of both parasite genotype and host factors in the pathogenesis of DL.

Keywords *Leishmania braziliensis*; DISSEMINATED LEISHMANIASIS; CUTANEOUS LEISHMANIASIS; MONOCYTES; TOLL LIKE RECEPTORS; INFLAMMATORY RESPONSE

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P3-065: ESTABLISHMENT OF A PRIMARY HAMSTER MACROPHAGE SYSTEM TO STUDY SUSCEPTIBILITY TO *Leishmania donovani* INFECTION

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Macrophages are important innate immune cells that detect and eliminate invading microbes and instruct the immune system for appropriate adaptive responses. *Leishmania* resists macrophage cytolytic activities and exploit these cells as hosts for intracellular proliferation. Chronic infection in humans can be either asymptomatic or causing devastating immune-pathologies. The host determinants and immune mechanisms underlying this dichotomy are largely unknown, even though pathways controlling the infection could inform on urgently needed, immune-therapeutic interventions. Mice are reported to control *Leishmania (L.) donovani* infection conversely to hamsters that develop progressive and lethal visceral leishmaniasis. Therefore, we propose to exploit these two rodent systems to investigate the role of *Leishmania*-macrophage interactions in disease outcome. C57BL/6 mice and Golden Syrian hamsters were used as source of primary macrophages. Peritoneal macrophages (PMs) were recovered from the peritoneal cavity and bone marrow-derived macrophages (BMDMs) were generated from precursor cells. Hamsters bone marrow precursor cells were cultured during 6 days in different culture media and in presence of different sources of CSF1 (macrophage colony-stimulating factor 1) to determine the best condition to generate fully differentiated macrophages. The macrophage phenotype was assessed by (i) microscopic analysis of morphology and capacity to phagocytose zymosan particles, and (ii) real time quantitative PCR (qRT-PCR) to monitor the expression of macrophage and polarization markers. Susceptibility of mouse and hamster PMs and BMDMs to *L. donovani* infection was tested using promastigotes freshly derived from hamster-isolated splenic

amastigotes. We first demonstrated by *in vitro* infection that only hamster but not mouse peritoneal macrophages allow robust proliferation of *L. donovani* infection, supporting our initial hypothesis that the different disease outcome observed in the corresponding animal models may be determined by the initial *Leishmania*/macrophage interaction. The heterogeneity of peritoneal exudate cells and the limited number of macrophages recovered per animal primed us to establish a protocol for hamster BMDM differentiation to obtain bulk quantities of highly homogenous cells for downstream transcriptomics analyses. Hamster progenitor cells cultured in presence of human recombinant CSF1 or fibroblast-conditioned medium differentiated into cells that were morphologically equivalent to peritoneal macrophages and showed high phagocytic capacity as judged by the uptake of fluorescent zymosan particles into lysotracker-positive vacuoles by more than 98% of these cells. The macrophage phenotype of these cells was further confirmed by increased expression of macrophage markers compared to undifferentiated progenitor cells, including CD68, CD14 and CSF1r. The low expression level of IL-10, ARG1 and TNFα further indicated that our protocol largely produces non-polarized (M0) macrophages. In conclusion, we established a robust protocol allowing the production of hamster bone marrow-derived macrophages. Preliminary infection assay confirmed the susceptibility of these cells to *L. donovani* promastigotes, thus validating our experimental system. We are currently conducting comparative transcriptomics analyses of *L. donovani*-infected hamster and mouse BMDMs with the aim to identify novel host pathways underlying the dichotomy in infection outcome observed in these animals. Our analyses have the potential to define innate macrophage responses that cause resistance to infection and thus may be exploited for future immune-therapeutic intervention.

Keywords *Leishmania donovani*; ANIMAL MODELS; BONE MARROW DERIVED MACROPHAGES; qRT-PCR; HOST-PATHOGEN INTERACTION

P3-066: PARASITE LOAD IN BALB/C EXPERIMENTAL INFECTION WITH *Leishmania* (*V.*) *guyanensis* / *L.*(*V.*) *shawi* HYBRID PARASITE IS HIGHER THAN OF THEIR PARENTAL STRAINS

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The genetic mechanisms involved in hybridization events in the genus *Leishmania* sp. have been extensively investigated. However, little is known about the effects of such events on the biology of hybrid strains. The present work aimed to determine the evolution of infection through variation of parasite load in skin lesion and lymph nodes over time for a hybrid strain of *L.* (*Viannia*) *guyanensis*/*L.* (*Viannia*) *shawi* (MHOM/BR/2001/M19672) and its comparison with the experimental model of the parental species *L.* (*V.*) *guyanensis* (MHOM/BR/1975/M4147) and *L.* (*V.*) *shawi* (MHOM/BR/1984/M8408). Parasite load was estimated in isogenic BALB/c mice after intradermal inoculation of *L.* (*V.*) *guyanensis*/*L.* (*V.*) *shawi* hybrid strain (n=5) and parental species, *L.*(*V.*) *guyanensis* (n=5) and *L.* (*V.*) *shawi* (n=5). Mice were infected with 10^6 stationary phase promastigotes. Skin lesion and draining lymph node parasite load were determined at 2, 7, 14, 21, 28, 35 and 60 days postinfection (PI) via a limiting dilution assay. The variation profile in the evolution of infection for each group was then traced

through a multiple linear regression analysis using the R software. The parasitic load curve during the evolution of infection was more similar between *L. guyanensis* and *L. shawi* parental strains with no significant differences among them (d.f.=1, t=0.002, p=0.99), which was characterized by stabilization in the parasitic load from the 35th day. The *L. (V.) guyanensis/L. (V.) shawi* hybrid showed a significant increase in the parasite load from the 35th day (d.f.=5,97, F=38.01, p<0.01, r^2=0.6) which differed from the parental species infection profile over time. The variation in the profile of the hybrid lineage represented a change in behavior, thus expressing phenotypic variability. This finding indicates that important changes can occur from a single recombination event, even when the parental species are similar to each other, thus opening a range of possible investigations from hybrid lineages in the *Leishmania* genus.

Keywords HYBRID STRAIN; *Leishmania* (*viannia*) spp.; PARASITE LOAD; EXPERIMENTAL INFECTION; BALB/C

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P3-071: IDENTIFICATION OF CD4⁺ T LYMPHOCYTES RESTRICTED EPITOPES OF *Leishmania mexicana* ELONGATION FACTOR-1α

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Leishmaniasis comprises a group of diseases caused by intracellular parasites of *Leishmania* genus. There are three clinical forms: Cutaneous (CL), Mucocutaneous (ML) and Visceral (VL) Leishmaniasis. In Mexico, CL is mainly caused by *L. mexicana*, being endemic in eighteen states. In 2010, our research group using immunonoproteomic analysis reported five "new" antigens of *L. mexicana* reactive to sera of CL confirmed patients. Afterward, the more prominent antigen p29 was identified as the Elongation Factor-1alpha (EF-1α), designed as EFLm. Although the key role of EFLm in host-parasite relationship is unknown; in VL caused by *L. donovani*, your homologous molecule (EFLd) can display also a non-canonical function as virulent factor, disrupting the Leishmanicidal effector mechanism in macrophages, evoking the SHP-1 activation that turn off the JAK/STAT signaling pathway inhibiting the NO generation increasing the intracellular survival of *Leishmania*, defining to the EFLm as potential pharmacological target. This scenario suggests the relevance to begin the study of the immunobiology mediated by EFLm in CL by this reason our objective was the identification Th lymphocytes epitopes, analyzing Th cytokine profiles related with protective or permissive mechanisms in leishmaniasis. Therefore, we starter with the recombinant protein production of EFLm (rEFLm), fused to H6-tag domain and purified by metal affinity chromatography. BALB/c mice were immunized with rEFLm. We evaluated the anti-EFLm specific IgG levels using an ELISA, as sensor of Th indirect response activation. Th specific response was determined by CFSE lymphoproliferation assay, using magnetic column purified CD4⁺ T

lymphocytes, activation marker CD44-PerCP and macrophages RAW264.7 as APC. The EFLm Th epitopes I-A^d restricted were identified by prediction based on 4 pockets: 1 (degenerate), 4 (aliphatic), 6 (A), 9 (A/S), and confirmed *in silico*. BALB/c mice were immunized with synthetic epitope and Th specific response was also evaluated by CFSE lymphoproliferation assay. This epitope was used as vaccine candidate in experimental LC by challenging BALB/c mice with $1x10^7$ *L. mexicana* promastigotes. At 4 weeks post-infection, the Th response was evaluated by lymphoproliferation assay and sera of experimental groups of mice were collected. As a results, rEFLm was purified and visualized by 12% SDS-PAGE, evidencing a band of ≈63 kDa corresponding to the fusion protein. The specific IgG levels anti-rEFLm increased 10 times compared with negative control and pre-immune mice. The prediction shown 2 possible epitopes, only one could be synthesized by chemicals restrictions. Our I-A^d restricted epitope was designed as EFp434: SSGGKVTKAATKAAKK. By *in silico* analysis, we obtained a low percentile range (≈2%), suggesting a higher binding affinity for I-A^d. Th specific lymphoproliferation was evidenced in both, rEFLm and EFp434 immunized mice. In experimental CL, also we observed an enhanced Th lymphoproliferative response and protective Th1 cytokine profile (IFN-γ, IL-2 and IL-6), suggesting a more detailed analysis as a vaccine candidate. In conclusion, rEFLm is a biotechnological tool that evokes humoral and cellular immune responses. The predicted EFp434 epitope, also evokes a potent Th protective response, which can be involved in the possible resolution of experimental CL. However, a more detailed study should be done.

Keywords EPITOPE; ELONGATION FACTOR-1 ALPHA; *Leishmania mexicana*

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P3-072: PARASITISM AND HISTOPATHOLOGICAL FEATURES OF SPLEEN, LYMPH NODES AND SKIN IN PATIENTS WITH RELAPSING VISCERAL LEISHMANIASIS AND AIDS.

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Visceral leishmaniasis (VL) is characterized by fever, splenomegaly, hepatomegaly and anemia. *Leishmania* amastigotes can infect mononuclear phagocytes of spleen, liver, bone marrow and lymph nodes (LN). Similarly, spleen and LN are critical reservoirs for maintenance of HIV, contributing to the virus persistence. Patients with VL co-infected with HIV/AIDS often remain with relapses, multiple hospital readmissions, and low quality of life despite drug therapy. We have previously reported the benefits of splenectomy as rescue therapy in patients with chronic drug-refractory VL. Herein, we described the parasitism and anatomopathological features of spleen, LN and skin in patients with VL-HIV/AIDS who underwent laparoscopic splenectomy. This study aims contribute to the understanding of host-parasite interactions, disease progression, reactivation and VL-

transmission. A total of 10 patients donated their spleen, and three patients also donated samples from mesenteric LN and skin from the incision site. They were confirmed to VL and HIV infection, not responsive in two cycles of Amphotericin B with six months interval, submitted to secondary prophylaxis (Amphotericin B biweekly) and adherent to highly active anti-retroviral therapy. After chirurgical procedure, spleens were weighted and 0,3-cm^3 splenic tissues sections, 0,5-cm^3 skin sections, complete mesenteric LN were collected and fixed in an alcoholic acid formalin solution. Tissues were paraffin-embedded, and sections were smeared with hematoxylin-eosin prior to microscopy examination. Structural changes of the spleen were systemically classified in three categories. Spleen type 1: well-organized white pulp with discernible peri-arteriolar lymphocyte sheath, germinal center, mantle zone and marginal zone. Spleen type 2: slightly disorganized white pulp with either hyperplastic or hypoplastic changes leading to a loss in definition of the limits between white pulp regions. Spleen type 3: moderately to extensively disorganized white pulp with poorly discernible regions. Follicular structure hardly distinct from the red pulp and T-cell areas. Lymph nodes and skin were evaluated for the presence of parasites, hemosiderin deposits (siderosis) and cellularity. Spleen weigh ranged from 295 g to 1882 g, with mean of 794.3 g and median 650 g. Three patients (30%) had spleen type 1, five (50%) spleen type 2 and two (20%) spleen type 3. All spleens had massive presence of *Leishmania* amastigotes. Three mesenteric LN had intense presence of parasites and one perisplenic LN was negative for *Leishmania*. Remarkable, two out of three skin samples had perivascular macrophages containing intense amastigote parasitism. Additionally, one patient had post kala-azar dermal leishmaniasis after splenectomy, with confirmed skin parasitism. Siderosis was present in all LN and four spleens. Disorganization of the spleen compartments is associated with more severe VL-presentation. Combine with the increasing of spleen size, the loss of lymphoid tissue architecture leads to a defective humoral and cellular response. Heavily parasitized mesenteric LN indicates its importance to the parasite persistence after treatment. Cutaneous parasitism infections may act as reservoirs contributing to disease reactivation and allowing anthroponotic transmission. Abnormal tissue retention of iron may underlie sideropenic anemia. We showed that the disorganization of splenic microenvironments

is relevant to human-VL, thus splenectomy may improve the clinical course of patients with chronic drug-refractory VL.

Keywords SPLEEN, LYMPH NODES; SKIN; PARASITISM, HISTOPATHOLOGICAL FEATURES

P3-073: FIRST REPORT OF *LEISHMANIA (MUNDINIA) MARTINIQUENSIS* IN SOUTH AMERICAN TERRITORY AND CONFIRMATION OF *LEISHBUNYAVIRUS* INFECTING THIS PARASITE

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Leishmaniasis are, in general, zoonotic infections affecting humans and several domestic and sylvatic animals, causing disease in some of them. Although domestic dogs are the main reservoir in urban areas, several studies have searched for other vertebrate animals which can play a role in the transmission cycle of *Leishmania* spp. Among the investigations that look for new possible reservoirs, there were reports on equids showing clinical manifestations of cutaneous leishmaniasis. The importance of researching *Leishmania* spp. infections in horses is due to the fact that these animals, as well as dogs and cats, live in close contact with humans. However, epidemiological data related to equine leishmaniasis or *Leishmania* parasites infection in horses are scarce, being most of the time presented only as a clinical case report. Studies carried out in Europe, Africa, and South America showed horses (*Equus caballus*), donkeys (*Equus asinus*), mules (*Equus asinus caballus*) and ponies (*E. caballus*) are parasitized by different *Leishmania* species, such as *Leishmania braziliensis*, *Leishmania infantum* and *Leishmania martiniquensis*. The species *L. martiniquensis* was first isolated from human patients with CL on the Martinique Island in 1995 but named in 2014. More recently, this species was assigned to the subgenus

L. (*Mundinia*), including other species such as *L. enrietti*, *L. macropodum* and *L. orientalis*. There are still few studies on these species, but one interesting factor is their presence worldwide and their capacity to infect different hosts. Some reports are showing *L. martiniquensis* infecting horses in different parts of the World. Here we describe for the first time a female horse living in Rio de Janeiro, Brazil, presenting cutaneous lesions in the left ear that were positive for *Leishmania* spp. after direct parasitological exam by microscopic examination histopathological examinations and parasite isolation in culture medium. The parasite cultivated was submitted to Multilocus Enzyme Electrophoresis for typing, but the profile was not conclusive. DNA from the obtained culture was submitted to PCR assay targeting ITSrDNA region followed by sequencing, and it was identified as *L. martiniquensis* after BLAST search (Identity= 97.95%; E value= 1,00E-163). Considering the recent description of *Leishbunyavirus* (LBV) infecting *L. martiniquensis*, we performed the protocol for LBV identification as previously described. Our results confirmed the presence of LBV in the *L. martiniquensis* strain isolated from this horse in Brazil. It is important to mention that the animal did not travel outside the Country but participated in championships in different Brazilian regions, some being endemic for visceral leishmaniasis associated with *L. infantum*. There were other horses in the horse breeding farm, but only this one presented cutaneous lesion and infection by *L. martiniquensis*. The clinical profile of the disease and its rapid healing or spontaneous cure may indicate that skin lesions related to Leishmania infection in horses is underdiagnosed. The animal showed complete remission of the lesion after three months of topical treatment (organophosphate and carbamate) and use of oral anthelmintics (metrifonate).

Keywords *Leishmania martiniquensis*; Leishbunyavirus; LBV; LEISHMANIASIS EPIDEMIOLOGY

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P3-074: CLINICAL, PARASITOLOGICAL, AND IMMUNOLOGICAL FEATURES DURING *Leishmania (Viannia) braziliensis* AND *L. (L.) amazonensis* INFECTION IN THE HAMSTER MODEL

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American tegumentary leishmaniasis (ATL) is an infectious disease caused by a protozoan belonging to the *Leishmania* genus, which compromises the skin and mucosa. Cutaneous leishmaniasis (CL) is the most common clinical presentation, but the clinical and immunopathological features can differ depending on the *Leishmania* species. Because of this, the knowledge of ATL physiopathology has to focus on each *Leishmania* species. *Leishmania (Viannia) braziliensis* is the most spread parasite in Brazil, causing not only CL but also mucosal leishmaniasis (ML), a more severe clinical form of the disease. *Leishmania (L.) amazonensis* is less frequent than *L. braziliensis*, but leads to spectral clinical presentation ranging from CL to diffuse cutaneous leishmaniasis (DCL). Of course, parasite antigens exert important role in the pathogenesis once *L. braziliensis* is associated with a hyperactivity of immune system, while *L. amazonensis* antigens downmodulates the effector response. Since most strains of mice are resistant to infection by species of the subgenus *Viannia,* which are responsible for most cases of CL in the Americas, our group has been studying the course of *L. braziliensis* infection in the hamster (*Mesocricetus auratus*), which has been considered a suitable model for CL. The hamster develop a progressive CL disease, sharing many clinical and histological features with human disease. In this work, we

investigated in the hamster model the clinical and immunopathogenic profiles associated with *L. amazonensis* or *L. braziliensis* infection. For this, hamsters were infected in the paw with 1 x 10^5 promastigotes of *L. braziliensis* or *L. amazonensis*. The lesions of *L. amazonensis* infected hamsters were nodular, non-ulcerated and significantly smaller (1.75mm) than those infected by *L. braziliensis* (2.35mm), which were ulcerated and with visible tissue damage. On the other hand, parasite load on the paw (7,4 x 10^7 parasites/gram of tissue) and on the lymph node (2.6 x 10^6) in the *L. amazonensis* infected group were higher than in the group infected by *L. braziliensis* (paw = 9.0x10^4 and lymph node = 2.0x10^4). The levels of IgG and mainly IgG2 anti-*Leishmania* from the group infected by *L. amazonensis* were higher than that observed in the group infected by *L. braziliensis.* Also, a significant increase in the expression of iNOS, IFN-ɣ and TNF was observed in the paw of *L. braziliensis* infected hamsters whereas, in those infected by *L. amazonensis* an increase in the arginase expression was observed. The reduced expression of effector cytokine genes observed in hamsters infected with *L. amazonensis*, in addition to the higher arginase, seems to be related to the elevated parasite load besides. On the other hand, the sustained capacity *L.braziliensis* antigens in inducing both regulatory, anti- and pro-inflammatory gene cytokines, could explain the predominance of ulcerated lesions and the reduced parasite replication observed in hamsters infected with *L.braziliensis*. These results demonstrated that hamster model for *L. amazonensis* and *L. braziliensis* infection presents clinical, parasitological and immunological aspects observed in human disease caused by these species.

Keywords *Leishmania braziliensis; Leishmania amazonensis*; HAMSTER MODEL; CLINICAL; IMMUNOPATHOGENESIS

P3-076:ARSENITE PROMOTES MACROPHAGE INFECTION BY *Leishmania donovani* THROUGH INDUCTION OF HO-1

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Visceral leishmaniasis due to *Leishmania donovani* is a major health problem in the Indian subcontinent, where it co-localizes with a mass intoxication by inorganic arsenic. Arsenite (As^{III}) has been shown to modify phenotype of human macrophages. This study aimed at evaluating the impact of sub-cytotoxic concentrations of As^{III} on the response of macrophages infected with *L. donovani*. Monocytes were isolated from PBMCs of healthy human donors, and differentiated into macrophages with M-CSF 50 ng/mL during 6 days, before to be exposed to As_2O_3 1 µM during 48h. Arsenite-exposed macrophages were then infected with *L. donovani* promastigotes at the 1:10 ratio. Arsenite significantly induced expression of M1 surface markers (CD80, TLR2, TLR4, $p<0.05$) and DC-SIGN ($p<0.01$), and repressed expression of M2 surface markers (CD206 and CD36, $p<0.01$ and $p<0.05$ respectively) of non-infected macrophages. Multiplex dosage of cytokines in the cell culture supernatant showed that arsenite decreased the secretion of both pro-inflammatory (TNF-α, IFN-γ) and Th2 and anti-inflammatory (IL-4, IL-1RA, CCL17) cytokines by infected macrophages ($p<0.05$). RT-qPCR profile was consistent with repression of both pro-inflammatory (IL-1β, TNF-α) and anti-inflammatory cytokines (IL-1RN) in infected macrophages. Transcription of HO-1 gene, known to be favorable to *Leishmania* intracellular multiplication, was induced in all donors ($p<0.01$). Slide cultures showed that arsenite increased proportion of macrophages with *L. donovani* compared to untreated control (mean fold change = 2.0±2.1, $p<0.001$). We hypothesized that parasite survival was favored by arsenite

through HO-1 induction, as it has been described to decrease pro-inflammatory response and oxidative burst. Slide cultures of untreated and arsenite-treated macrophages were exposed to either zinc protoporphyrin IX (ZnPP IX), a HO-1 inhibitor, or α-lipoic acid (ALA), a thiol-containing antioxidant protecting against arsenite-induced oxidative stress. Treatment of macrophage with these compounds neutralized the effect of arsenite, as infection rate was similar to untreated condition (mean fold change = 1.2±0.3 for ZnPP IX and 1.6±0.8 for ALA, not significant). Altogether, these results showed that arsenite-induced oxidative stress is responsible for HO-1 induction which favors parasite survival. Arsenite also modifies macrophages phenotype by decreasing their cytokinic response. This implies that environmental exposure to sub-cytotoxic concentrations of arsenite could be an aggravating factor for *L. donovani* leishmaniasis.

Keywords ARSENITE; *Leishmania donovani*; MACROPHAGES

P3-077: INFLUENCE OF *LEISHMANIA* INFECTION ON THE BIOLOGY OF HOST CELLS

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Many parasitic diseases occur with a sex-bias in terms of incidence, morbidity and mortality towards male individuals. Leishmaniasis is such an example as it presents with significantly higher number of cases in male patients in various endemic areas. However, this phenomenon cannot be explained by cultural or behavioural determinants only, indicating that biological factors can alter the susceptibility towards an infection and influence the outcome of the disease. Besides sex steroid hormones, such as testosterone or estradiol, chromosomal factors (XX, XY) can affect the host immune response and the parasite respectively. *Leishmania* parasites can survive and multiply in innate immune cells, such as macrophages while suppressing an effective clearance of the parasite. To understand biological sex differences in leishmaniasis, the present study is aimed to analyse the influence of the host sex in the infection of macrophages by *Leishmania* ssp. on a cellular and transcriptional level. Therefore, an *in vitro* model for the infection of primary human macrophages was developed and optimised using an automated confocal high content screening (HCS) system (Opera Phenix™). $CD14^+$ monocytes were isolated from buffy coats of healthy human blood donors following differentiation into macrophages and infection by the viscerotropic *L. infantum* strain 3511. All experiments were performed in parallel with macrophages from female and male donors. Visualisation of intracellular *Leishmania* parasites was enabled by immunofluorescent staining, in which the infected cells were stained with DAPI and an antibody against the highly abundant parasite HSP90. Samples were taken at different timepoints post infection and total macrophages, parasite burden and infection rates were measured by HCS. Analysis is done

with a customised image analysis algorithm integrated in the Harmony software. For the transcriptomic approach, RNA was isolated from infected macrophages as well as respective controls and subjected to next generation sequencing. According to the chosen multiplicity of infection (MOI) a time-dependent course of the infection could be observed. Furthermore, the initial phagocytosis rate did not differ between the sexes, within the first 6 hours post infection (hpi) but already after 24 hpi, the infection rates were higher in macrophages from male donors compared to female donors. These differences remained over the entire time course of infection. As determined by a significant reduction in numbers of parasites per infected cell, the clearance of the parasite appeared more efficient in female macrophages. Transcriptional dynamics of sequenced RNA will be examined for sex-specific differences in molecular mechanisms during infection. The sex-specific differences in infection with *Leishmania* could be successfully reproduced in an *in vitro* infection assay. Further analyses, such as cytokine profiles, will hopefully provide further insight into the molecular basis of the differences.

Keywords HUMAN PRIMARY MACROPHAGES; *IN VITRO* INFECTION; SEX DIFFERENCES

P3-078: INFLUENCE OF HOST NUTRIOME ON IMMUNOLOGICAL CONTROL OF *LEISHMANIA* INFECTION

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Immunologic control of parasitic infections arises from a combination of humoral and cellular mechanisms, both of which may be influenced by host nutritional status. Micronutrient depletion or over-repletion impairs the functioning of the immune system, potentially resulting in increased susceptibility to and poor immunologic control of protozoal infections. We aim to synthesize the knowledge surrounding the interplay between host micronutrient status and tissue-based protozoal infections. Leishmaniasis is a tissue-dwelling parasitic infection in which disease severity is determined by the host's immune system. Research suggests that acquired factors such as nutritional inadequacies play a significant role in immunosuppression and enhanced pathogenicity. Five electronic databases were searched with combinations of search terms from database inception to March, 2022. A total of 10,334 articles were retrieved, however after a deduplication step only 8348 articles remained. Screening was performed independently by two reviewers with discrepancies arbitrated by a tertiary reviewer. After title/abstract screening, 206 articles were full text screened, leaving 12 eligible for absolute inclusion. Following screening, a comprehensive bias

assessment will be carried out using the GRADE approach. Interim findings suggest that malnourished individuals are at greater risk of acquiring a significant leishmanial infection. Deficiencies reported thus far include malnourishment in general, vitamin A, zinc (n=3 each), iron (n=2), fiber, vitamin E, potassium, selenium, and copper (n=1 each). Disruptions to immune cell count (n=3), and antibody levels (n=1) were also noted. The data will be summarized to systematically map published literature that will illuminate several ways in which nutrient deficiencies or abnormal micronutrient status alter and impair immune function in persons with leishmaniasis. This synthesized body of information will ultimately inform adjunctive therapeutic decisions in the context of leishmaniasis, which has the potential to improve patient prognosis.

Keywords NUTRIOME; LEISHMANIASIS; SYSTEMATIC REVIEW

P3-078.1: Identification of adipocytes as target cells for *Leishmania infantum* parasites

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Leishmania infantum is the causative agent of visceral leishmaniasis transmitted by the bite of female sand flies. The recommended drugs for treating leishmaniasis include Amphotericin B. But over the course of the years, several cases of relapses have been documented. These relapses call into question the efficiency of actual treatments and raises the question of potential persistence sites. Indeed, *Leishmania* has the ability to persist in humans for long periods of time and even after successful treatment. Several potential persistence sites have already been identified and named 'safe targets'. As adipose tissue has been proposed as a sanctuary of persistence for several pathogens, we investigated whether *L. infantum* could be found in this tissue. Experiments were approved by the ethics committee of the Nice School of Medicine, France (Protocol number: 2017-56). *In vitro* and *in vivo* experiments were performed with Recombinant *L. infantum* - expressing the Green Fluorescence Protein and *L. infantum* - expressing the Luciferase reporter, respectively. Mouse and Human adipocytes were infected, and PCR, histology, microscopy and electron microscopy were used to follow the infection. *In vitro*, we demonstrated that *L. infantum* were able to infect mousse (Figure 1) and human adipocytes. Adoptive transfer experiments allowed us to demonstrate that *Leishmania* parasites were alive inside adipose tissue and had the capacity of infecting naive mice.

Altogether our results suggest adipocytes as a 'safe target' for *L. infantum* parasites. Treatment with poor access to the adipose tissue would be poorly effective at resolving the infection and would likely be followed by relapses with parasite exiting the adipocytes. In this context, it would be interesting to define new drugs against *L. infantum* with penetration into adipocytes to efficiently target parasites within these cells.

Keywords: *Leishmania*; ADIPOCYTES; 'SAFE TARGET'

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P4-031: GENOME-WIDE IDENTIFICATION OF T-CELL EPITOPES FOR VACCINE DESIGN AGAINST *LEISHMANIA* (*VIANNIA*) SPECIES

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Leishmania is a protozoan parasite causing several disease presentations collectively known as leishmaniasis. Pathogenic species of *Leishmania* are divided into two subgenera, *L.* (*Leishmania*) and *L.* (*Viannia*). Species belonging to *L.* (*Viannia*) have only been reported in Central and South America. These species predominantly cause cutaneous leishmaniasis, but in some cases parasites can migrate to the nasopharyngeal area and cause a highly disfiguring mucocutaneous presentation. Despite intensive efforts, no effective antileishmanial vaccine is available for use in humans, although a few candidates designed for *L.* (*Leishmania*) are now in clinical trials. After sequencing the genome of *L.* (*Viannia*) *panamensis* we noticed a high degree of sequence divergence among orthologous proteins from both subgenera. Consequently, some of the vaccine candidates that have been designed for *L.* (*Leishmania*) may not work properly for *L.* (*Viannia*) species. To help in vaccine design, we looked for $CD4^+$ and $CD8^+$ T-cell epitopes in the proteins of four genomes available for *L.* (*Viannia*) species. Prediction was performed with three independent bioinformatic tools, using the most frequent human MHC class I and class II alleles in the affected geographic area. Molecular docking simulation was also used to model binding of candidate peptides to selected MHC alleles. Binding of the most promising peptides to specific alleles was experimentally verified by using surface plasmon resonance (SPR). We found millions of peptides predicted to bind to the selected alleles, but only few of them could be classified as promiscuous on the basis of allele coverage. Sequence similarity searches corroborated that, due to sequence divergence, approximately half of previously published candidates for *L.* (*Leishmania*) species are not present in *L.* (*Viannia*) proteins. Our prediction

algorithm was validated with those epitopes present in *L.* (*Viannia*), with 85-100% accuracy on average. All the prediction data generated in this study are publicly available in an interactive database called VianniaTopes. Binding of a subset of the most promiscuous peptides was experimentally confirmed by SPR.

Keywords *Leishmania Viannia* subgenus; EPITOPE PREDICTION; VACCINE DESIGN

P4-032: IMMUNOMODULATORY ACTIVITY IN VITRO OF A MIXTURE OF TRITERPENE HEDERAGENIN GLUCOSIDE SAPONINS AND CHROMANE HYDRAZONE AS POSSIBLE MECHANISM OF ACTION OF CROMALEISH®, A POTENTIAL LEISHMANICIDAL DRUG

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The hederagenin glucoside saponins (SS) and chromane hydrazone (TC2) combined in a 1:1 ratio have high potential in antileishmanial therapy since both compounds alter the survival of Leishmania and the ability to infect adjacent macrophage. Given the importance of immunological factors in the response to treatment, it is important to target compounds that eliminate the parasite, but also intervene in inflammatory processes. In this study, the in vitro immunomodulatory activity of the mixture of SS and TC2 against infection of human monocyte-derived macrophages (huMDM) with Leishmania braziliensis was evaluated according to nitric oxide production by flow cytometry and the production of MIP-1□, TGF-□1, and IL-4 by ELISA. The TC2:SS mixture induced the increase of MIP-1a and decrease of IL-4 with respect to uninfected huMDM. Moreover, the immunomodulatory profile was corroborated by cell activation by pseudopod elongation. The leishmanicidal effect of the TC2 and SS mixture was verified by the decrease in the percentage of infected cells (15.5%) compared to infected and untreated cells (48.5%) and the decrease in parasite load (3 parasites/cell) in infected and treated cells while 7 parasites/cell were found in untreated cells. MIP-1 α mediates immune cell chemotaxis, which promotes inflammatory responses. Because MIP-1α binds to the CCR1 receptor which seems to have multifaceted roles in injury, including significant input in reepithelialization during the wound healing process, the induction of MIP-1 production by the TC2:SS mixture suggests a possible mechanism of action for the mixture of SS and TC2 in the resolution of L. braziliensis infection.

Keywords CUTANEOUS LEISHMANIASIS; MCP-1α; IL-4; *Leishmania braziliensis*

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P4-034: IMMUNIZATION WITH CENTRIN-DEFICIENT *LEISHMANIA BRAZILIENSIS* DOES NOT CONFER PROTECTION AGAINST SUBSEQUENT INFECTION

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Leishmaniasis affects millions of people in major areas of the globe. Despite this disease burden, a vaccine remains unavailable. Previous infection by *Leishmania* induces robust immunity against subsequent disease, indicating that vaccination is an attainable goal. Recent advances in this field have shown that immunization with *Leishmania* lacking *Centrin* confers robust protection against challenge. *Centrin* is a calcium-binding cytoskeletal protein involved in centrosome duplication. Centrin deficiency in *Leishmania* causes arrested division at the amastigote stage, resulting in an attenuated cell line, unable to cause disease upon experimental infection. Herein, we employed the LeishGEdit toolbox to generate a Centrin-deficient *Leishmania braziliensis*, the causative agent of mucosal and disseminated disease. Firstly, we generated a transgenic cell line expressing Cas9 and T7 RNA polymerase, which was later employed for the targeted deletion of Centrin. Whole-genome sequencing of centrin-deficient *L. braziliensis (LbCen$^{-/-}$)* did not indicate the presence of off-target mutations. *LbCen$^{-/-}$* promastigotes displayed normal *in vitro* growth whereas axenic and intracellular *LbCen$^{-/-}$* amastigotes showed a multinucleated phenotype with impaired survival following macrophage infection. Upon experimental infection of BALB/c mice with *LbCen$^{-/-}$* promastigotes, lesions failed to develop whereas parasites were detected two weeks later at the inoculation site. Parasites became undetectable after five weeks of infection, indicating impaired survival of *LbCen$^{-/-}$ in vivo*. Surprisingly, mice immunized with

LbCen$^{-/-}$ and challenged with *LbWT* parasites were not protected as lesion development and parasite multiplication were observed. On the contrary, mice that healed a primary infection with wild-type *L. braziliensis* were successfully protected, failing to develop lesions and displaying a significantly lower parasite load, compared to control mice. These results indicate that immunization with the attenuated *LbCen*$^{-/-}$ does not protect against a challenge infection, differently from results reported for other species. We hypothesize that the effector immune response induced by *LbCen*$^{-/-}$ is not robust or adequate to prevent disease development. In conclusion, the complexity of the immune response against *Leishmania* sp. highlights differences regarding protective immune responses, and indicates that investigating these discrepancies shall contribute to advances in the field of vaccine development.

Keywords LEISHGEEDIT; LEISHMANIASIS; GENETIC MANIPULATION; ATTENUATION; VACCINE DEVELOPMENT

P4-035: EXTRACELLULAR VESICLES ISOLATED FROM *Leishmania major* PARASITES CONFER PROTECTION IN MICE AFTER EVs-VACCINATION TRIAL

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Extracellular Vesicles (EVs) are membrane-derived structures that can be released by a wide range of organisms and can transport molecules like proteins, ADN, ARN, metabolites, among others. EVs have been described to be involved in cellular communication with the capability to induce changes in recipient cells at a physiological level. Since EVs "mirror" the cell of origin, it has been proved in laboratory conditions that they can confer a protective state against pathogens, activates pro-inflammatory response, and transfer virulence across pathogen strains. Leishmania parasites can secrete exosomal proteins in a non-conventional way and mimicking the heat shock that the parasite undergoes during infection, it is possible to obtain EVs under laboratory conditions. The virulent factor Gp63 has immunomodulatory capacities in the context of macrophage infection. Moreover, it has been shown that the release of EVs by the parasite during the bloodmeal intake from the sandfly is related to an augmented skin lesion and the release of proinflammatory cytokines. In this research, we tested the capacity of *L. major* derived EVs to confer protection against leishmaniasis infection. EVs were isolated from *L. major* gp63$^{-/-}$ (KO) and *L. major* WT culture after heat shock treatment and analyzed with protein quantification, NanoSight NS300 analysis (NTA), electron microscopy (TEM) and LC-MS/MS. Subcutaneous and intraperitoneal vaccine trials were performed on C57BL/6 mice using EVs-WT or EVs-gp63$^{-/-}$ with or without CpG, and PBS control, prior to challenge infection in the right hind footpad with *L. major* WT ($5x10^6$ promastigotes). Disease progression/protection were monitored by measuring footpad swelling weekly with a metric caliper up

to 10 weeks post-infection. In total, 644 proteins were identified using LC-MS/MS with 514 common proteins, 44 unique proteins for EVs-WT and 86 unique proteins for EVs-gp63$^{-/-}$. After the immunization, a significant lower footpad swelling was observed in the EVs-WT and EVs-gp63$^{-/-}$ groups, compared with the control group. However No significant difference in inflammation was observed in groups vaccinated with WT or GP63 exosomes neither in groups vaccinated with the same type of exosomes but with or without CpG. Extracellular vesicles have demonstrated the capacity of biological molecules transmission, being of special interest in vaccine approaches as vehicles for antigen delivery. These results demonstrated that an EVs-based vaccination approach maintained a lower inflammation in-vivo after infection with *L. major*. Further research is required for understanding the mechanisms involved in the protection observed with the EVs-vaccination approach.

Keywords EXTRACELLULAR VESICLES, *Leishmania*, IMMUNE RESPONSE, VACCINE

P4-037: A SYSTEMATIC REVIEW ON THE USE OF TETRAMERS FOR ISOLATION OF ANTIGEN SPECIFIC B CELLS AMONG LEISHMANIASIS INFECTED HOSTS

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Assessment of specific responses to leishmaniasis remains a challenge to health care especially in poor resource settings in Africa. Protective Immune responses to the *Leishmania* infection are TH1 dependent, however marked hypergammaglobulinemia has been noted in active VL patients. Thus the need to investigate the role of specific B cells in this infection. However, this is hindered by few B lymphocytes in a heterogenous population. This led to development of tetramers to improve sensitivity of detection of antigen specific B cells. The aim of this review is to collect evidence in order to determine the extent of the use of tetramers as a tool in identifying antigen specific B cells among *Leishmania* infected individuals. Full text indexed publications and original research done to characterize antigen specific B cells were included. Studies done in both human and mouse models were included basing on their population, intervention, control and outcome (PICO) approach. We defined a search strategy and explored different electronic databases to identify publications, short communications, review articles and theses that involved work with tetramers. Using predefined key words together with Boolean operators, we created search strings in the PubMed NLM advanced search. Articles generated were downloaded and uploaded into the Rayyan website. Here in, relevant articles were filtered out and thereafter imported into referencing software. Twenty-one articles containing information about 5 different *Leishmania* antigens were included i.e. *Leishmania major* LACK antigen, Aspartate transcarbamylase, Lipophosphoglycan 3, *Leishmania donovani* uridine 5′-monophosphate synthase and phosphoenolpyruvate carboxykinase (PEPCK) peptide. It was noted that the *Leishmania* antigens used which were enzymes existed as

tetramers and these were used to identify specific CD4+T cells. There was paucity of information on tetramer use for B cells in *L. donovan*i infection. Using tetramers is one of the techniques proven for characterisation of antigen specific B cells. However, application of this technique during VL infection has not been adequately evaluated. There is need to expand characterisation of antigen specific B cell responses using tetramers. This is important in determining potential candidate antigens for vaccine designs.

Keywords LEISHMANIASIS; TETRAMERS; ANTIGEN SPECIFIC B CELLS

P4-038: ENGINEERING A SAND FLY-BASED PAN-*LEISHMANIA* VACCINE FOR HUMANS

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Leishmaniasis is a spectrum of diseases transmitted by sand fly vectors via the deposition of *Leishmania* spp. parasites together with saliva, into the host's skin during blood feeding. A vaccine for human leishmaniasis is considered an achievable goal; however, we still do not have an anti-*Leishmania* vaccine for humans available today, a consequence (amongst other factors) of a lack of pre-clinical to clinical translatability. Pre-exposure to uninfected sand fly bites or immunization with defined sand fly salivary proteins was shown to negatively impact infection, giving relevance to the use of defined sand fly salivary proteins as anti-*Leishmania* vaccines. Still, cross-protection reports are rare and dependent on the phylogenetic proximity of the sand fly species; in other words, the applicability of a sand fly saliva-based vaccine will probably be limited to a defined geography, one parasite species and one form of leishmaniasis. Additionally, some sand fly salivary proteins have potent bioactive properties, which may pose a barrier considering the safety requirements for approval of a human pharmaceutical. To address these limitations, as a proof of principle of a future pan-*Leishmania* vaccine, in this study we engineered, through a reverse vaccinology approach that maximizes translation to humans, a

"broad spectrum" sand fly saliva-based anti-*Leishmania* vaccine. First, we predicted, both murine and human MHC-I and MHC-II restricted epitopes (iedb.org; consensus method) in the context of two distinct sand fly salivary proteins previously studied in the context of anti-*Leishmania* vaccines - PdSP15 from *Phlebotomus duboscqi* and LJL-143 from *Lutzomyia longipalpis* - to uncover their "immunogenic map" and the overlaps considering the two species. Then we selected cross-species immunogenic regions of these two proteins (excluding at least partially the residues important for their bioactivity) and fused them in-tandem to generate a single neo antigen. The *in silico* analysis was validated ex vivo, through T cell proliferation experiments, which showed that the fusion protein (administered as a DNA vaccine) maintained the immunogenicity of both PdSP15 and LJL143. Interestingly, while no significant effect was detected in the context of *L. major* transmission by *P. duboscqi*, this DNA vaccine was defined as partially protective, in the context of *L. major* transmission by *L. longipalpis* sand flies. Importantly, a high IFNγ response alone was not enough to confer protection, which mainly correlated with low T cell mediated *Leishmania*-specific IL-4 and IL-10 responses, and consequently with high pro/anti-inflammatory cytokine ratios. Overall, our immunogenicity data suggest that to design a potentially safe sand fly-based pan-*Leishmania* vaccine, without geographic restrictions and against all forms of leishmaniasis is an achievable goal. Of note, as a proof-of principle, we believe our approach has the potential to be applicable not only to the anti-*Leishmania* vector-based vaccines' field, but also to other branches of knowledge that require the design of multi-epitope T cell vaccines with a higher potential for translation.

Keywords Anti-*Leishmania* VACCINE; SANDFLY SALIVARY PROTEINS; CUTANEOUS LEISHMANIASIS; VISCERAL LEISHMANIASIS; TRANSLATION

P4-039: EVALUATION OF THE LAAG VACCINE ASSOCIATED TO CAF FAMILY ADJUVANTS BY INTRANASAL ROUTE AGAINST MURINE CUTANEOUS LEISHMANIASIS

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Leishmaniasis is an infectious disease caused by parasites from genus *Leishmania* and transmitted by sand fly. Clinical manifestation presented as lesions in the cutaneous region, which can progress to more severe manifestations and cause deformations in the skin. The chemotherapy is extremely toxic to the patient and there is no vaccine approved for human use. Thus, it shows the need of a search for an effective vaccine. The association of studied vaccine as whole antigens of *Leishmania amazonensis* (LaAg) vaccine with new adjuvants could be an alternative to discovery a new vaccine formulation. Adjuvants are substances that are associated to vaccines to enhance or modulate the immunogenicity of the antigen present in the formulation increasing dencritic cell capture and maturation to promote an effective T cell response. CAF adjuvants (CAF01) are cationic liposomes that increase the phagocytosis by Dendritic cells. Besides, CAF can be formulated with MPLA (CAF06) or Poly I:C (CAF09) with capacity to activate TLR4 and TLR3, respectively. Our focus is to evaluate the ability of adjuvants (CAF01, CAF06 and CAF09) associated with whole antigens of Leishmania amazonensis (LaAg) by intranasal route to improve immunogenic and protective responses against infection caused by *L. amazonensis*. The BALB/c mice were vaccinated twice by intranasal route before the infection. The animals were divided in different groups treated with the respective vaccines (LaAg, LaAg+CAF01, LaAg+CAF06, LaAg+CAF09) and control group (PBS). After the vaccination, $2x10^5$

promastigotes of the parasite were inoculated in the right footpad of the animals and the lesion growth caused by the infection was measured weekly by pachymetry. The parasite load of the infected organs was determined by the limiting dilution analysis (LDA). The results indicated that LaAg+CAF06, LaAg+CAF09 vaccines weren't able to enhance the protection in comparison to LaAg. We observed that mice vaccinated with LaAg plus CAF01 was able to enhance the control of lesion and parasite load in comparison to LaAg. Our results indicate a potential use of the intranasal route for vaccination using LaAg plus CAF01. Studies to optimize the formulation and to understand the protection mechanism are in progress.

Keywords VACCINE, *L.amazonensis*; ADJUVANT; LIPOSOMES; BIOTECHNOLOGY

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P4-040: HOST-DIRECT THERAPIES TARGETING *LEISHMANIA* PARASITES

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Leishmania has developed mechanism to disrupt both innate and adaptive immune response, allowing intracellular parasite proliferation and long-term and persistent infections in the mammalian host. The high attrition rate during drug discovery campaigns and the use of combination therapies to avoid the development of resistances, may indicate the need to explore alternatives based on host-parasite interactions. In this regard, the induction of programmed cell death could remove the niche for infection[1], and simultaneously expose parasites to the immune system[2]. In this scenario, parasites could be more susceptible to antimicrobial agents or the activation of immune system could eliminate intracellular parasites. Recently, some studies have shown that the induction of pyroptosis in bone-marrow macrophages infected with *L. amazonensis*, far from removing the infection, contributed to its spread. Herein, we wonder whether other programmed cell death pathways such as apoptosis or necroptosis could help in achieving these goals. Bone-marrow derived macrophages infected or not with *L. donovani* were exposed to different combinations of drugs known for induction of intrinsic or extrinsic apoptosis and necroptosis pathways. Host cell death was measured by a commercial LDH release assay kit and parasite proliferation by infrared fluorescence emission as readout. Our results confirmed that when the death is associated to the formation of a pore in the cell membrane which occurs in necroptosis, parasites were viable and free to spread to other cells. However, this is a very simplistic system without the presence of other important host-cells as dendritic cells and lymphocytes. In order to test our hypothesis in a more physiological environment, we have performed the same experiments in *ex-vivo* splenic

cultures where uninfected cells were activated by the presence of parasite antigens and damage-associated molecular patterns and then incubated with infected *ex-vivo* splenic cultures. Under these conditions there were a significant reduction in parasite viability and infectivity towards naïve macrophages.

Keywords *Leishmania*; EX-VIVO SPLENIC EXPLANT; PROGRAMMED CELL DEATH; APOPTOSIS; NECROPTOSIS

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P4-042: THE USE OF IMMUNOSUPPRESSIVE AGENTS CAN INFLUENCE THE TREATMENT OF VISCERAL LEISHMANIASIS WITH GLUCANTIME®

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The increasing use of immunosuppressive agents to treat autoimmune diseases has sharply risen the risk of visceral leishmaniasis (VL), the most severe clinical form of this parasitic disease. Immunosuppressed patients exhibit less response to VL treatment, traditionally mediated by antimonials, and are more likely to relapse when the immunosuppressive treatment is reintroduced after VL cure. The main objective of this study was to evaluate the efficacy of VL treatment under immunosuppressive conditions in an experimental model of VL. BALB/c mice were immunosuppressed at clinical doses with TNF antagonist (anti-TNF) or methotrexate (MTX), and a non-immunosuppressed control group was included. The immunosuppression conditions were maintained along the experiment. The animals were subsequently infected with 10^7 *Leishmania infantum* promastigotes and after 6 weeks of infection (6W), Glucantime® was administered following standard recommendations. After nine weeks of experiment (9W), parasite load was evaluated by quantitative PCR in liver, spleen and bone marrow. Levels of specific IgG against the parasite were determined by ELISA. In order to evaluate the cellular immune response, flow cytometry was used to measure the expression of specific pro-inflammatory cytokines by $TCD4^+$ and $TCD8^+$ lymphocytes, and Cytokine Bead Assay was used to determine the levels of Th1, Th2 and Th17 cytokines in the supernatants of cultured splenocytes after soluble *Leishmania* antigen (SLA) stimulation. Although no significant differences in parasite load were observed, results showed that animals of the immunosuppressed group with MTX had a greater splenomegaly, as well

as a higher number of cells in the spleen compared to the other groups, which means that they seemed to develop a VL that did not resolve after treatment. In addition, this group showed a significant proliferation of $CD4^+$ and $CD8^+$ T lymphocytes producing IFN-γ. Similarly, this group showed a slight increase in $CD4^+$ T cell exhaustion mediated by the expression of PD1. Although specific lgG levels in this group remained unchanged during 6W, they slightly increased after Glucantime® administration, which is related to a worst response to antimonials treatment if MTX is used as immunosuppressive agent. On the other hand, the specific cell response of the anti-TNF group and the control one was similar. Additionally, it was found that in both control and anti-TNF groups, specific IgG titters increased during 6W of infection and decrease after three weeks of Glucantime® treatment, indicating an adequate response to VL treatment in these groups. In conclusion, this work shows that the immunosuppressives treatments used to treat some autoimmune diseases influence the efficacy of Glucantime®, needed for the resolution of visceral leishmaniasis.

Keywords VISCERAL LEISHMANIASIS; IMMUNOSUPPRESSION; GLUCANTIME

P4-044: PROTEIN VACCINE TARGETS FOR VISCERAL AND TEGUMENTARY LEISHMANIASIS

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About 20 species of *Leishmania* cause approximately 1 million tegumentary and 30000 visceral leishmaniasis new cases around the world annually. Although immunization of the population would be an efficient control alternative, so far there are no human leishmaniasis vaccine available. Our group demonstrated that *L. (Viannia) naiffi* antigens induce well-modulated responses and that sera from cured cutaneous leishmaniasis volunteers recognized soluble antigens considered immunodominant. Other experiments demonstrated that hamster's intranasal immunization with total antigens of *L. (V.) naiffi* and *L. (Leishmania) amazonensis* induce protective immunity against *L. (V.) braziliensis* infection. Thus, this work aimed to identify the immunodominant proteins present in the soluble fractions of the total antigen of *L. (L.) amazonensis* and *L. (V.) naiffi*, more conserved within the genus *Leishmania*, as candidates to compose a pan specific vaccine for the control of leishmaniasis. The soluble antigens were sub fractionated on a polyacrylamide gel and the molecular weight fractions between 35 and 100KDa were extracted and analyzed by mass spectrometry for proteomic identification. The most abundant proteins were analyzed for similarity with host proteins. Epitopes were predicted by B lymphocytes recognition, by high-affinity ligands to HLA class I and II molecules, by

promiscuity among HLA alleles, and by low similarity to human proteins (<30%). A sub proteome with 328 validated proteins was obtained, of these, 128 presented low similarity value to human, dog and hamster proteins. Sixteen more immunodominant proteins were identified in terms of the number of epitopes with high binding affinity to BCR and high promiscuous to HLA I and II. The homology analysis allowed the identification of 11 proteins with the most orthologs among seven *Leishmania* species. This work demonstrated the potential of these proteins as promising vaccine targets capable of inducing a humoral and cellular pan-specific immune response in humans, which may in the future contribute to the control of leishmaniasis.

Keywords LEISHMANIASIS; PAN SPECIFIC VACCINE TARGETS; IMMUNODOMINANT EPITOPES

Financing The present work was carried out with the support of the National Council for Scientific Development and Technological (CNPq) and Oswaldo Cruz Foundation

P4-046: DIVERGENT SUSCEPTIBILITY TO ANTIMONY AND FREQUENCY OF TREATMENT FAILURE IN CUTANEOUS LEISHMANIASIS CAUSED BY *L. (V.) PANAMENSIS* SUBPOPULATIONS.

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The relationship between *in vitro* parasite susceptibility to anti-leishmanials and the clinical response to treatment of cutaneous leishmaniasis remains unclear. Innate immune responses of host macrophages to infection, along with drug-mediated modulation of these responses, may influence treatment outcome and assessment of drug susceptibility. This relationship has not been considered in the evaluation of drug susceptibility in cutaneous leishmaniasis. To define the relationship between drug susceptibility and antimony treatment outcome, we determined antimony susceptibility of 39 *L. (V.) panamensis* strains from the corresponding patients with documented response to treatment and absence of known risk factors for treatment failure such as chronic lesions, non-adherence to treatment, immunosuppressive co-morbidity or treatment, and age-related pharmacokinetic alteration. To understand the contribution of host cells to drug susceptibility, we comparatively evaluated the anti-leishmanial activity of antimony (SbV) in various host cell models: *ex vivo* in primary human monocyte-derived macrophages isolated from healthy donors and peripheral blood mononuclear cell cultures, and *in vitro* using the promonocytic U937 cell line. Zymodeme profile was assessed in these parasites to identify subpopulations of *L. (V.) panamensis* previously associated with intrinsic resistance (zymodeme 2.3) or sensitivity (zymodeme 2.2) to antimony. Cells were infected with stationary-phase promastigotes, and exposed to 32 µg SbV/ml for 72 hours at 34°C. Parasite burden was quantitatively determined by RT-PCR of *Leishmania* 7SLRNA, and normalized with the human PPIB gene. We found that treatment

outcome in this study population was not associated with susceptibility in any of the host cell models; however, based on this study and previous studies, we observed that the infection with parasites pertaining to zymodeme 2.3 resulted in a higher rate of treatment failure, with 59% (23/39) of patients infected with zymodeme 2.3 failing treatment, whereas 38% (12/32) of patients infected with zymodeme 2.2 failed. Comparative analysis of drug susceptibility in different cellular models showed that clinical strains that belong to zymodeme 2.3 were resistant to SbV in the U937 cell line, but displayed significantly greater susceptibility to SbV in primary macrophages ($p < 0.0001$) and PBMCs ($p = 0.0019$). In contrast, sensitive strains (zymodeme 2.2) displayed comparable susceptibility to SbV in both primary host cells and the U937 macrophage model. These results demonstrate that the potency of antimony for strains presenting intrinsic resistance to this drug, is enhanced in primary macrophages compared to the U937 promonocytic cell line. These findings suggest that parasite factors that influence susceptibility to antimony participate in the response to treatment in patients with CL. The higher frequency of failure of treatment with antimony in patients infected with *L. (V.) panamensis* of zymodeme 2.3, underscores the need for alternative therapies, and also provides a unique opportunity to probe and understand the bases of susceptibility to antimony.

Keywords LEISHMANIASIS; HOST CELL MODELS; SUSCEPTIBILITY; TREATMENT OUTCOME; ANTIMONY

Financing This work was financed by the NIH/NIAID Tropical Medicine Research Centers (TMRC) grant U19AI129910

P4-047: HYPERCORTICOIDISM INDUCED DURING EXPERIMENTAL VISCERAL LEISHMANIASIS IS RELATED TO UP-REGULATION OF ACTH RECEPTOR IN THE ADRENAL AND INCREASED IL-6 LEVELS

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Visceral leishmaniasis (VL) is a neglected tropical disease, caused by protozoan *Leishmania (Leishmania) infantum* in the New World. The protozoan antigens and host immune response are known to be closely involved in the clinical course of VL. Several infectious diseases are associated with hypothalamic-pituitary-adrenal (HPA) axis disorders by elevating circulating glucocorticoids (GCs), which are known to have an immunosuppressive potential. Previous studies in VL-golden hamster model, demonstrated that *L. infantum* infection was associated with increased levels of systemic cortisol. The cortisol levels were correlated with hematological, biochemical, and immunological parameters of VL severity. In this work, we investigated the putative influence of *L. infantum* infection on the mechanisms driving glucocorticoid production by adrenals. Male BALB/c mice were infected intraperitoneally with 2 x 10^7 *L. infantum* promastigotes and the animals were followed-up at 5, 30, and 120 days post-infection (dpi). The animals were treated with adrenocorticotropic hormone (ACTH) to address the reactivity of the adrenal ACTH receptor (MC2R) in vivo. In comparison to non-infected animals, we found that *L. infantum*-infected mice showed increased levels of plasma corticosterone at

5 dpi and a peak of hormone production at 30 dpi, in parallel with the rise of parasite burden in the spleen. Nevertheless, the circulating levels of corticosterone decreased at 120 dpi, despite the high parasitic load in the spleen. The detection of MC2R in the adrenals of infected mice presented a similar profile. Indeed, as was observed for corticosterone, the IL-6 production showed a bell-shaped profile with an increase at 30-dpi, but decreasing at 120-dpi. At 30 and 120 dpi, the injection of ACTH was associated with an increase of corticosterone levels in both uninfected mice and infected mice. However, despite presenting reduced MC2R receptor at 120 dpi, adrenals from *L. infantum*-infected mice had a higher capacity to increase of corticosterone after ACTH stimulus (2.8-fold) than non-infected ones (1.9-fold). It demonstrate that adrenal function is preserved in infected mice during all time. Together, our data suggested that the increased adrenal MC2R expression and circulating IL-6 levels are important to control the hypercorticoidism during the course of *L. infantum* infection. In summary, our results showed that experimental VL in mice evolve with hypercorticoidism since the early time after infection, probably not as a direct effect of leishmanial antigens on adrenal glands, but rather a consequence of the immunopathological events related to the *L. infantum* infection. Thereby, *L. infantum* infection-induced hypercorticoidism at the initial phase can be responsible, at least partly, to the progression of the *L. infantum* infection.

Keywords *L. infantum;* VISCERAL LEISHMANIASIS; HYPERCORTICOIDISM; IL-6, ACTH RECEPTOR

5.6 OMICS - MOLECULAR BIOLOGY – BIOCHEMISTRY - OTHERS

P1-070: SIGNALING PATHWAYS AND TRANSCRIPTIONAL CHANGES INVOLVED IN THE EARLY LIVER INFECTION BY *Leishmania infantum* IN BALB/C MICE

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Leishmania infantum infection in mice triggers transcriptional changes not only in the parasite but also in the target organs. The evaluation of gene expression *in vivo*, from tissue of the affected organ, allows understanding the immunological pathways and transcriptional changes involved in the development of early infection in mice. High-throughput real-time qPCR was used to evaluate the expression of 223 genes related to immunometabolism at different timepoints (1,3,5 and 10 days post infection) of *L. infantum* infection from liver samples of infected BALB/c mice and control mice. Three parameters were studied: parasite load, liver weight and gene expression profile. A multivariate Principal Component Analysis (PCA) was performed to identify gene expression patterns in mice. Gene set enrichment analysis (GSEA) was performed and STRING interaction

network was constructed; topological clustering algorithm was applied to determine clusters and functional enrichment analysis was performed. 1-day infected mice showed a gene signature characterized by the expression of Principal Component 2 (PC2) correlated genes. Gene set enrichment analysis applied to PC2 correlated genes revealed the overrepresentation of *Interferon Signaling pathway* (FDR = 0.039) with the upregulation of *Stat1*, *Irf1*, *Irf7*, *Irf5*, *Icam1* and *Chemokine receptors bind chemokines pathway* (FDR = 0.199) with the upregulation of *Cxcl10*, *Cxcl9*, *Xcl1*, *Ccl2*, *Ccr5.* The detailed analysis of gene interactions showed that after partition of the Protein-Protein Interaction (PPI) STRING network, two main clusters were formed. The functional enrichment analysis showed Gene ontology (GO) biological processes overrepresented by genes in cluster A: *T cell selection* (FDR= 1.13E-6), *regulation of tolerance induction* (FDR= 7.34E-6) and *inflammatory response to antigenic stimulus* (FDR= 2.06E-5). GO Biological processes overrepresented by genes in cluster B: *Response to cytokine* (FDR= 1.31E-23), *Chemokine-mediated signaling pathway* (FDR=1.29E-15), and Jak-STAT signaling pathway (FDR= 4.75-5) as KEGG pathway. All of the genes in this analysis were positively correlated to PC2, however *Rxra* was negatively correlated to PC2 and downregulated. The downregulation of transcriptional factors (*Rxra, Pparg, Nr1h3*), was consistent with the upregulation of proinflammatory markers (*Ifng, Nos2, Tnf*). In spite of the upregulation of proinflammatory markers, the upregulation of regulatory markers (*Pd-l1, Pd-l2, Tim3*) suggested that inflammatory response is dampened down and unable to tackle parasite proliferation. In conclusion, this massive high-throughput real-time qPCR gene expression analysis shows that *L. infantum* induces strong regulation of the immune response in liver from a very early timepoint of infection, allowing for the establishment of the parasite. The gene signature 1 day post infection was mainly characterized by the upregulation of mediators involved in interferon signaling and cell chemotaxis. The inflammatory response is not effective to control parasite replication due to the activation of regulatory mechanisms. The transcriptional induction of pro-inflammatory genes is also supported by the downregulation of transcriptional factors involved in lipid metabolism and inflammation. Se agradece la financiación concedida por La Universidad de La Laguna (ULL), la cofinanciación por la Agencia Canaria de Investigación, Innovación y Sociedad de la Información de la Consejería de

Economía, Conocimiento y Empleo y por el Fondo Social Europeo (FSE) Programa Operativo Integrado de Canarias 2014-2020, Eje 3 Tema Prioritario 74 (85%).

Keywords TRANSCRIPTIONAL PROFILING; HIGH-THROUGHPUT RT-qPCR; GENE EXPRESSION; EARLY INFECTION; *Leishmania infantum*

P1-071: CHARACTERIZATION OF *LEISHMANIA* SPP. CAUSING CUTANEOUS LESIONS WITH A NEGATIVE PARASITOLOGICAL DIAGNOSIS IN PANAMÁ

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Cutaneous leishmaniasis (CL) is a zoonosis caused by parasites of the genus *Leishmania*, it is transmitted through the bite of sandflies. This infection is characterized by presenting mainly dermal involvement. The parasitological diagnosis of CL is based on the microscopic detection of amastigotes in skin scrapings from a lesion or promastigotes isolation in culture. However, these direct tests can be negative, even in cases where the clinical/epidemiological features suggest that the individual has the infection. The results of the direct diagnosis of CL can be influenced by many factors such as: parasite genetic characteristics, evolution of the lesion, parasitic load (PL) and technical expertise. Between 2015 and 2019, a total of 342 direct tests for CL with a negative parasitological diagnosis were registered at the Instituto Conmemorativo Gorgas de Estudios de la Salud, Panama. Of these, 179 (52.3%) were positive when a conserved region of the kDNA minicircle was amplified in a routine PCR test. The main objective of this study was to characterize the *Leishmania* parasites present in CL lesions with a negative parasitological diagnosis but with positive PCR tests. Eighty-two samples of lesions with negative scrapings/cultures for *Leishmania* parasites, but positive by a PCR that amplifies a sequence of the Hsp-70 gene (1286pb) of *Leishmania* sp. were evaluated. These PCR products were used for the identification of the species and genetic variants of *Leishmania*, through two different methods. A restriction fragment length

polymorphism (RFLP-Hsp-70) assay, using HaeIII and BccI enzymes and a Sanger sequencing DNA analysis, to identify the species by a phylogenetic method. Of the 82 positive samples by PCR-Hsp-70, 69 (84.2%) were characterized at the species level by RFLP-Hsp-70. According to this analysis, 41 samples (59.4%) corresponded to *Leishmania (Viannia) panamensis* and 28 (40.6%) resulted *L. (V.) guyanensis*. The phylogenetic analysis showed that all obtained sequences were grouped into subgenus *Viannia*, 21 of these sequences corresponded to *L. (V.) panamensis*. However, 11 were grouped with the sequence of a genetic variant previously described in Panama as *Leishmania* sp.1. Knowing the PL in CL lesions is important for the eventual clinical follow-up of the patient, including the response to treatment. A low PL can affect the parasitological diagnosis, due to potential false negative results. Characterizing the species and genetic variants of *Leishmania* associated with this kind of lesions with low PL has a particular diagnostic, clinical, and epidemiological interest. This study confirms that *L. (V.) panamensis* is frequent in this type of lesions, however a genetic variant (*Leishmania* sp.1) was also detected in an important proportion (40.6%) of the evaluated samples. These findings should be considered during the diagnosis and clinical management of CL in Panama.

Keywords *Leishmania (viannia) panamensis*; PARASITOLOGICAL DIAGNOSIS; PARASITE LOAD; MOLECULAR CHARACTERIZATION; PANAMA

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P1-072: MOLECULAR IDENTIFICATION OF *Leishmania* FROM STORED GIEMSA STAINED SLIDES

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Honduras is an endemic region for cutaneous and visceral leishmaniasis that affects infant and adult populations depending on the clinical presentation. Identification of *Leishmania* species in clinical samples is key for prognosis, surveillance, and guiding control strategies. However, the main diagnosis method in most endemic regions is direct microscopy, which does not provide species identification. In this study, we evaluated the performance of molecular methods for the identification of *Leishmania* from stored Giemsa slides collected between 2017 and 2020 at the University Hospital in Tegucigalpa, Honduras. Collected specimens from patients with clinical suspicion of leishmaniasis were put onto microscope slides, fixed with methanol, dried at room temperature, and stained with Giemsa (1:20 dilution) for 20 minutes. Slides were examined by microscopy at 1000X and stored at room temperature until shipped to NAMRU-6 in Lima, Peru for molecular testing. Stained slides with more than one amastigote in 300 microscopic fields were scraped and the tissue was suspended in 1X PBS. DNA was extracted and used for molecular detection of *Leishmania* using a 70bp kDNA-PCR specific for the *Viannia* subgenus and a 120 bp kDNA-PCR that amplifies the *Viannia* and *Leishmania* subgenus. *Leishmania* species were determined by a FRET probes-based Nested Real-Time PCR based on melting curve analysis using control DNA from seven *Leishmania* reference species. Samples from 92 patients were collected (eight bone marrow aspirates, 71 smears, 13 imprints) with a mean age of 17.8 years and 51% female. More than 58% of the cases came from the regions of Choluteca, El

Paraíso and Francisco Morazán. Microscopy detected 67 positives (three visceral, three mucosal, and 61 cutaneous) out of which 64 were subjected to PCR testing. Using the combined results of both kDNA-PCR assays, we detected 53 positives for *Leishmania spp.* (82%). However, the FRET probes-based Nested Real-Time PCR assay detected species in only three samples, two *L. (L.) infantum-chagasi* and one *L. (V.) braziliensis*. Few studies in the New World assess the potential use of stored Giemsa stained slides for molecular identification of *Leishmania*. Our results show the high sensitivity of kDNA-PCR to detect *Leishmania* DNA on slides collected and stored under field conditions five years ago. However, species identification remains a challenge due to the low number of nuclear DNA copies that are targeted by the Nested Real-Time PCR. Improving storage conditions of Giemsa stained slides and improved molecular methods could potentially overcome this limitation allowing for retrospective studies or leishmaniasis surveillance in remote endemic sites of Central and South America.

Keywords MICROSCOPY SLIDES; GIEMSA STAIN; MOLECULAR IDENTIFICATION; SPECIES DETECTION; HONDURAS

P1-074: *Leishmania donovani* SYSTEMS ANALYSIS REVEALS PROTEIN TURNOVER AS A KEY DETERMINANT IN PARASITE STAGE DIFFERENTIATION

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Leishmania survival and pathogenicity depends on the parasite's capacity to adapt to different host environments through stage differentiation of promastigotes within the sand fly, and of amastigotes inside mammalian host cells. *Leishmania* stage-specific expression occurs in the absence of classical transcriptional regulation, raising the question on alternative regulatory mechanisms. We investigated these mechanisms applying RNAseq, label-free quantitative proteomics and phosphoproteomics approaches on hamster-purified amastigotes and corresponding, culture-derived promastigotes. Comparison of the stage-specific transcriptomes and proteomes revealed a three times higher dynamic range for protein compared to RNA abundance suggesting that translational and post-translational mechanisms outweigh RNA turnover in regulating stage differentiation. We next investigated protein turnover by applying label-free quantitative proteomic on both amastigotes and promastigotes in presence or absence of the irreversible, proteasomal inhibitor lactacystin. Inhibitor-treated amastigotes were viable but failed to convert into promastigotes in culture, revealing an essential role of protein degradation in *Leishmania* development. We identified 180 proteins (fold change ≥ 2, adj. p-value < 0.01) as proteasomal targets during the amastigote-to-promastigote transition, which may represent putative differentiation factors. Applied on

promastigotes, lactacystin treatment rescued 289 proteins from degradation (fold change ≥ 2, adj. p-value < 0.01) but neither affected parasite morphology nor proliferation. Interestingly, we observed stabilization of amastigote-specific proteins in lactacystin-treated promastigotes (and vice versa) suggesting a role of proteasomal degradation in regulating stage-specific protein abundance. Surprisingly, 18 proteins (fold change ≥ 2, adj. p-value < 0.01) were stabilized in both stages, including 11 proteins that were only identified in lactacystin treated parasites, thus uncovering a set of proteins that undergo constitutive degraded in our experimental system. Our data identified respectively 6 and 11 protein kinases that were rescued from degradation in treated amastigotes and promastigotes, suggesting differential protein kinase turnover as a regulatory switch in parasite development. Finally, we investigated the pathways regulated by protein kinase activities during differentiation by label-free, quantitative phospho-proteomics analysis of splenic amastigotes and culture-derived promastigotes. We identified 7095 phosphopeptides in promastigotes and 2080 in amastigotes of which 6128 (61%) are exclusive to one stage or the other. Twenty five proteins with exclusive stage-specific phosphorylation were linked to proteasomal protein degradation including 3 proteasomal subunits, 5 ubiquitin transferases, 5 ubiquitin ligases, 1 ubiquitin-conjugating enzyme, 1 ubiquitin-activating enzyme and 10 ubiquitin hydrolases. In conclusion, our results link stage-specific, proteasomal degradation of protein kinases to parasite differentiation and vice versa link stage-specific protein kinase activities to differential phosphorylation of proteasomal components. This reciprocal relationship likely establishes a proteasome/kinome regulatory network that controls *Leishmania* stage differentiation and confirms both the kinome and the proteasome as interesting targets for anti-parasitic intervention.

Keywords *Leishmania donovani*; DIFFERENTIATION; PROTEIN TURNOVER; KINASE; PROTEASOME

P1-075: DEVH1 RNA HELICASE *KNOCK-IN Leishmania infantum* PROMASTIGOTES DOWN-REGULATE PARASITE SURVIVAL GENES

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Trypanosomatids and other eukaryotic organisms form granules to compartmentalize untranslated mRNA molecules and proteins involved in splicing, transcription, adhesion, and signaling. This gene expression regulation mechanism normally takes place under stress conditions. The compartmentalized mRNA molecules are regulated in the cytosol by RNA-binding proteins (RBPs), which form mRNA-protein complexes (mRNPs) present in the granules. Some RNA helicases are RBPs that unwind RNA and displace other RBPs. This is an ATP-demanding process. The putative ATP-dependent DEAD/H RNA helicase (DEVH1) from *Leishmania infantum* is one of such helicases. A previous study confirmed that DEVH1 is present in cytoplasmic granules. The DEVH1 encoding gene is annotated in the *L. infantum* JPCM5 genome sequence (TriTrypDB). The objective of this research is to get insight into the biological roles of DEVH1 using a knock-in *L. infantum* promastigote line. DEVH1 knock-in parasites were generated using the pTEX trypanosomatid expression plasmid vector. The selection agent was geneticin at 100µg/mL. pTEX-DEVH1 knock-in and pTEX transfection control promastigotes were grown in axenic culture to the stationary phase. Total mRNA was isolated, and cyanine-labelled first-strand cDNA was synthesized. Whole genome shotgun DNA microarray hybridization analysis was performed. Gene Ontology (GO) terms were ascribed to genes with TriTrypDB, and GO enrichment analysis was

performed to obtain a differential gene expression overview. The GO term enrichment analysis supports that several genes down-regulated in DEVH1 knock-in promastigotes are involved in resistance to redox and biotic stress, including evasion of the immune response related to the host's complement system. The glutamine aminotransferase (GLS), the tryparedoxin 1 (TryX), and type II glutathione peroxidase-like tryparedoxin peroxidase (TrxP) genes are down-regulated in DEVH1 knock-in promastigotes. GLS yields glutamic acid for glutathione and trypanothione biosynthesis. TrxP is involved ROS detoxification, such as lipid-derived hydroperoxides in trypanosomatids. A concanavalin A-like lectin is also down-regulated in DEVH1 knock-in promastigotes. The GO biological process term "evasion of host immune response via regulation of host complement system" is associated to this gene. The amastin-like protein gene (LINF_080011900) is also down-regulated in DEVH1 knock-in promastigotes. Amastins were thought to be specific of the amastigote stage, although amastin mRNAs have been detected in metacyclic promastigotes, in agreement with the pre-adaptation hypothesis. Finally, the HASP/SHERP cluster is also down-regulated in DEVH1 knock-in promastigotes. The HASPA, HASPB, and SHERP proteins were previously described to be antigens and infective promastigote markers. qRT-PCR analysis has confirmed down-regulation of HASPA, SHERP, and HASPB. HASPA1 and HASPA2 have almost identical sequences and are not distinguishable by qRT-PCR. The HASPB TaqMan assay was designed in the inner gene region to exclude sequences overlapping with HASPA1, HASPA2, and SHERP. In conclusion, the DEVH1 knock-in promastigote line down-regulates several genes that are present in fully differentiated promastigotes or are related to ROS detoxification (HASPA2, HASPB, SHERP, amastin, concanavalin A-like lectin, trypanothione and tryparedoxin peroxidase), and up-regulate genes that may be related to processes triggered in less differentiated promastigotes or that slow down metacyclogenesis.

Keywords *Leishmania infantum*; DEAD/H RNA HELICASE; KNOCK-IN; DIFFERENTIAL GENE EXPRESSION

Financing RICET-RETICS (MICINN and FEDER)

P1-076: RNA BINDING PROTEINS AS TRANS-REGULATORS IMPACTING SURVEILLANCE AND INFECTIVITY IN *LEISHMANIA.*

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Leishmania spp. protozoan Kinetoplastids present peculiar gene expression fundamentally dependent upon post-transcriptional control. This elevates the importance of RNA binding proteins for gene regulation in these parasites. Building upon the mRBPome we isolated previously (Pablos, Ferreira et al., MCP, 2019), 70 mRNA-bound RBPs were selected from the three main *L. mexicana* lifecycle stages. A trans-regulator knockout clone library was created through barcoded CRISPR and screened for essential roles in cellular differentiation and macrophage or mouse infections. Of the 70 RBPs screened, 40 are essential to cell viability and 18 contribute to lifecycle progression to human-infective stages and/or parasite infectivity. Examination of individual knockout lines for amastigote-specific mRBPs showed normal promastigote growth dynamics, whereas infection of peritoneal macrophages was inhibited or ablated, suggesting essential roles of RBPs for amastigote viability and virulence. Immunoprecipitation of multiple mRBPs will identify associated transcript targets that may represent novel virulence factors.

Keywords *Leishmania;* RBP; RNA; KNOCKOUT; INFECTION

P1-079: LOSS-OF-FUNCTION SCREENS FOR DRUG MODE OF ACTION AND RESISTANCE MECHANISMS IN *LEISHMANIA*.

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The few drugs available against *Leishmania* are poorly understood in terms of mode of action (MOA) and their use is complicated by the emergence of drug resistance. We developed powerful genomic screens for deciphering MOA and resistance mechanisms for licensed and experimental drugs. Here, we describe our efforts with two loss-of-function screens. One deals with CRISPR-Cas9 where we constructed a plasmid library of six guide RNAs (gRNAs)/gene against the *Leishmania infantum* genome. This library has been transfected into parasites expressing Cas9 and first selected for growth in the presence of two concentrations of Amphotericin B (AMB). The gRNA vectors in cells growing with drugs were identified by next-generation sequencing (NovaSeq 6000). The AMB screen analysis showed an enrichment of three different gRNAs against the sterol 24-c-methyltransferase genes (SMT). This over-representation is steady through the three AMB biological replicates and gradual depending on the concentration of the drug. Targeted inactivation of the SMT gene confirmed its role in AMB resistance and validated the screen. The AMB screen also highlighted a hypothetical protein, and its genomic inactivation is in progress to confirm its role in the MOA of the AMB drug. We carried out additional Cas9 screens selecting for resistance to Miltefosine (MIL) and for the experimental drug GSK143295. The MIL screen highlighted the miltefosine transporter (MT1) but also a RING-variant domain protein and an ATP-binding cassette protein. The GSK143295 screen highlighted several candidates including the lorien protein and a CCR4 associated factor protein. The other loss-of-function screen being tested relies on an RNAi screen in *L. braziliensis*. We confirmed, as others, that a stem-loop strategy under the control of the ribosomal promoter is effective in knocking down the

expression of MT1 leading to MIL resistance. For whole genome approaches, we designed new constructs where 500 bp inserts are cloned in a cassette between two ribosomal promotors. Constructs integrated in the genome targeting MT1 led to MIL resistance. We are furthering this strategy with other genes and drugs, and are attempting to improve transformation efficiency in *L. braziliensis* to enable us to carry out whole genome RNAi screens. Our loss-of-function screens will refine our understanding of drug MOA and may reveal new drug targets.

Keywords LEISHMANIASIS ; LOSS-OF-FUNCTION SCREEN ; DRUG RESISTANCE ; CRISPR-Cas9 ; RNAi

P1-080: ESTIMATING COMPLEX AND MULTI-CLONAL INFECTIONS IN *LEISHMANIA* INFECTED PATIENTS AND RESERVOIRS

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Leishmaniasis a complex disease, comprising several parasite species, hosts, reservoirs, vectors and clinical symptoms. The clinical manifestations vary from mild cutaneous lesions to severe visceral damage, and genetic polymorphisms from both the host and the parasite have been associated with disease severity. Hybrids and genetic exchange between species and strains have already been reported for numerous parasite groups, including the *L. donovani* complex, where several species can cause visceral leishmaniasis. This has relevant implications in disease epidemiology, as it can fasten the spread of virulence and drug resistance genes. The presence of multiclonal infections, required for hybridization, could also aid in parasite adaptation to different hosts and stress conditions, as different sub-populations of the parasite could be selected in different scenarios. The presence of multiclonal infections have already been reported in some *L. donovani* isolates from the Indian subcontinent and Africa using WGS and in *L. infantum* dogs in Brazil using multilocus microsatellite typing. However, the extent of multiclonal infection across several geographic locations, parasite species and hosts have not been yet estimated. In the present work, we are exploring fluctuations in allele frequency of heterozygous SNPs positions in genome sequencing of Leishmania isolates as a measure of multiclonal infection. The main premise is that while in clonal infections heterozygous SNPs are expected to have similar read depths in both alleles, complex multiclonal infections will disturb this proportion. We are correcting SNP calls for several confounding factors as chromosomal somy, mapping quality, call quality and read depth variations. As different Leishmania species/populations have different levels of heterozygosity and were sequenced in different depths, clonal simulated isolates with the same

characteristics as each evaluated population were generated and used as a control. Preliminary results using ~450 whole genome sequencing of *L. infantum* and *L. donovani* isolates, from dogs and humans from Africa, Asia and Brazil have shown that a significant proportion of isolates from all sites and hosts appears to be multiclonal. We are planning on expanding this analysis to species from the L. viannia subspecies, to also evaluate multiclonal infections in cutaneous leishmaniasis. Finally, the proposed analysis will be packed in a framework, which could easily be adapted to other organisms.

Keywords MULTICLONAL INFECTION; *Leishmania*; SNPs

P1-081: MACROPHAGE SURFACE PROTEIN DISULFIDE ISOMERASE (PDI) AUGMENTS INFECTION BY *Leishmania (L.) amazonensis*

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Leishmaniases comprise a spectrum of diseases caused by protozoan parasites of the genus *Leishmania* spp., transmitted by the bite of infected female phlebotomines. With more than 350 million people at risk of infection worldwide, the estimated number of people infected with leishmaniasis per year ranges from 700,000 to 1 million. Infections by different *Leishmania* species may lead to tegumentary or visceral complications in humans. Chaperones present on macrophages surface play a fundamental role in the regulation of cellular homeostasis and may affect survival and infectivity of *Leishmania*. Protein disulfide isomerase (PDI) is one of the 20 most abundant chaperones of the endoplasmic reticulum (ER). The main PDI functions are oxidation, reduction and isomerization of disulfide bonds, with a canonical role of assisting in the isomerization of disulfide bonds in nascent ER proteins. The presence of PDI on the macrophage surface was associated with increased infection by *Leishmania (L.) chagasi*, a species associated with visceral leishmaniasis. The present study aimed to evaluate PDI role on the infection by *Leishmania (L.) amazonensis*, a species responsible for cutaneous leishmaniasis. Flow cytometry was carried out to confirm the presence of PDI on macrophage surface. Bone marrow derived-macrophages (BMDM) from BALB/c mice were then blocked with anti-PDI polyclonal antibody and infected with promastigotes of *Leishmania (L.) amazonensis*. BMDM from transgenic mice overexpressing PDI (confirmed by Western blot) and wild type macrophages were also *in vitro* infected with *L. (L.) amazonensis*. *In vivo* imaging using M2269 La-LUC infection in transgenic mice overexpressing PDI and wild type mice is ongoing for comparison of lesion swelling and parasite load. The results of flow cytometry indicated a low abundance of

PDI on macrophage surface. Infection of macrophages blocked with anti-PDI was lower compared with infection in the presence of isotype antibody. Accordingly, infection of transgenic macrophages overexpressing PDI was higher than of the wild type counterparts. We expect that *in vivo* infections will lead to higher lesion swelling and parasite loads in PDI transgenic mice in comparison with the wild type counterparts.

Keywords *Leishmania (L.) amazonensis*; PDI; MACROPHAGE INFECTION

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P1-082: TRANSFECTION OF *Leishmania mexicana* PROMASTIGOTES MEDIATED BY HEAT SHOCK

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The cell membrane autophagy process described in *Leishmania mexicana* during transformation from promastigote to amastigote in vitro entails a non selective mechanism for internalizing extracellular macromolecules or other sort of materials. Taking advantage of this cellular phenomena it is possible to incorporate, in a very fast and reproducible way, dissimilar substances into the cell, without affecting its survival. Latex beads, fluorescent or not (0.1um) were incorporated into the cells, when present in the growth medium, at the beginning of the differentiation process, as shown by the visualization of the matter by fluorescent microscopy and transmission electron microscopy. Also the plasmids pNUS and pLENTV2 added to the cell medium at the beginning of the differentiation process were incorporated inside the cells . The plasmid pNUS generates a transient transfection meanwhile the pLENTV2 was incorporated in the parasite genome, as revealed by the YFP fluorescence displayed in the whole promastigote body, mantained during several generations of cultured promastigotes. The method here described offer a way to study any parasite that differentiate in vitro under heat shock conditions.

Keyword *Leishmania mexicana*, DIFFERENTIATION; TRANSFECTION; HEAT SHOCK

P1-083: SEQUENCING AND CHARACTERIZATION OF THE KINETOPLAST GENOME OF *Leishmania infantum* PARASITES

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Leishmania parasites have a unique mitochondrium, a common characteristic to kinetoplastidae. It contains the kinetoplast, rich in DNA made of a complex network of interlocked circular molecules of two types: thousands of minicircles (800-900 bp) and a small number of maxicircles that encode for 6 mitochondrial genes and 12 cryptogenes. The kinetoplast is particularly relevant to the identification of parasite- specific biomarkers and potential drug targets. We aim here at elucidating the mitochondrial genome of two strains of *Leishmania infantum* species. Biological samples were obtained through in vitro promastigote culture of two *L. infantum* strains followed by mitochondria purification. Promastigotes were lysed in a dounce homogenizer in presence of a hypotonic buffer. After clearing of the homogenates at low speed, the supernatants were centrifuged at 17000g at 4°C and the pellets (corresponding to mitoplasts) were used to extract the kinetoplast DNA (kDNA) by phenol-chlorofom extraction and ethanol precipitation. HiFi SMRTBell® Libraries were prepared using SMRTBell Express Template Prep Kit 2.0. We used AMPure® PB Beads with a <3kb cut-off to preserve as much as possible of the lower fraction. Sequencing of the kinetoplast genome was performed using the PacBio-SMRT technique Sequel I. Sample & Library concentration and quality were assessed using FragmentAnalyzer (HS Large Fragment 50kb kit) and Qubit (Broad Range & High Sensitivity). Prior to mapping the reads with BWA-mem to the

Leishmania infantum reference genome, FastqC will be used for quality control. The mapped reads corresponding to the nuclear genome will be removed from the upcoming analysis. The unaligned reads will be extracted from the BAM files using Samtools view. All unaligned reads will then be processed with Trimmomatic. The remaining reads will be processed with SPAdes for De Novo assembly. Scaffolds larger than 500 nt will be used for maxicircle and minicircle BLAST analyses. Inventory of minicircle classes will be performed through multi-sequence alignments, predictions of the encoded gRNAs and potential assignment to maxicircle editing cascades. Identified minicircles will be annotated and clustered, and their copy numbers will be estimated. Maxicircles will be identified by alignment with other *Leishmania spp.* on GenBank using BLAST. Then they will be manually annotated by comparisons with other sequences available on GenBank including maxicircle sequences mainly from *L. infantum*. Open reading frames will be identified to predict features of encoded proteins and to identify novel biomarkers and molecular signatures. The present work will provide novel insights on the structure and content of the kinetoplast genome of *Leishmania* parasites. The used long-read technique of sequencing will enable a high quality of the data.

Keywords *Leishmania infantum*; KINETOPLAST DNA; LONG-READ SEQUENCING; MINICIRCLE; MAXICIRCLE

Financing Horizon2020 RI programs: EasiGenomics (GA824110), PHINDaccess (GA811034). MERST–Tunisia (LR16IPT04)

P1-084: SINGLE CELL TRANSCRIPTIONAL CHARACTERISATION OF *LEISHMANIA MAJOR* RETROLEPTOMONAD PROMATIGOTES

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Leishmania transmission occurs after the bite of an infected phlebotomine fly. During their passage inside the fly, *Leishmania* parasites go through several developmental stages that are morphologically and transcriptionally distinct. However, only metacyclic promastigotes can establish an infection in a mammal. Metacyclogenesis, the biological process leading to the formation of metacyclic parasites, was thought to be irreversible until retroleptomonad promastigotes were described. After every blood meal, the remaining metacyclic promastigotes de-differentiate to rapidly multiply and differentiate into metacyclic promastigotes, increasing the fly infectiousness. We performed the first scRNA-seq of a midgut-residing population of retroleptomonad promastigotes compared to a population of metacyclic promastigiotes of *Leishmania major*. Female *Phlebotomus duboscqi*, a natural vector of *L. major*, were artificially fed through a chicken skin membrane filled with rabbit defibrinated blood spiked with 1-2x10^6 *L. major* amastigotes/mL. Blood fed flies were separated after 2h and maintained at 26°C with 75% humidity until day 9 when they reached a mature infection with >75% metacyclic promastigotes (assessed by Phase-contrast microscopy counts of dissected midguts). A subset of those infected flies was fed on non-infected balb/c mice and brought back into the incubator for another 24h at 26°C with 75% humidity. Retroleptomonad-enriched promastigotes were recovered from twice fed flies while metacyclic-enriched promastigotes were recovered from day 10 single fed flies. Promastigotes were washed 3 times in PBS before being processed for single cell RNA sequencing using the 3' end kit from 10X

genomics. Two technical replicates for each sample were sequenced. Sequencing results were analyzed using a custom pipeline, similar to the Cell Ranger single-cell software, with optimization for the *L. major* genome. We successfully prepared single cell RNA-seq libraries to analyze 11, 454 *L. major* metacyclic and retroleptomonad promastigotes. Our preliminary data show, 1,449 metacyclic promastigotes constitute a transcriptionally homogenous population of cells in which classical metacyclic markers (SHERP, HASPa, HASPb) were highly expressed. By contrast, 10,005 retroleptomonad promastigotes displayed more transcriptional heterogeneity with the cells organized along two opposite expression gradients: (1) cell multiplication and (2) metacyclic specific gene expression and the retroleptomonad promastigote gene expression profiles were significantly distinct from those of metacyclic promastigotes. Those results contribute to our understanding of the previously proposed model for retroleptomonad biology.

P1-085: 3’ NUCLEOTIDASE/NUCLEASE IS INVOLVED IN MILTEFOSINE SUSCEPTIBILITY IN *Leishmania infantum* CLINICAL ISOLATES

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Visceral Leishmaniasis (VL) is a neglected tropical disease whose control relies primarily on chemotherapy. Nevertheless, current therapies have severe shortcomings, highlighting an urgent need for innovative safe and efficacious treatments, as well as understand the mechanism of resistance to preserve the current available drugs. Miltefosine has been used successfully to treat VL in India, but it was unsuccessful in a clinical trial for VL in Brazil (cure rate = 60%). Miltefosine treatment failure in visceral leishmaniasis in Brazil has been associated with deletion of the miltefosine susceptible locus (MSL) in *Leishmania infantum* (Carnielli et al., 2018 EbioMedicine 36:83-91). The MSL contains four genes: 3'-nucleotidase/nucleases LINF_310031200 (NUC1), and 3'-nucleotidase/nucleases precursor LINF_310031300 (NUC2); helicase-like protein LINF_310031400 (HLP); and 3,2-trans-enoyl-CoA isomerase LINF_310031500 (TEI). To investigate the role of each gene in susceptibility to miltefosine we engineered an MSL+ (MA01A-C1) *L. infantum* clone to express the T7 RNA polymerase and Cas9 endonuclease. Then, using CRISPR Cas9 technology we generated knockout cell lines for each gene in the MSL individually, Δ*nuc1*, Δ*nuc2*, Δ*hlp*, Δ*tei*, for both 3’-nucleotidase/nucleases, Δ*nuc1/nuc2* and for the whole MSL locus Δ*msl*. Deletion of both 3’nucleotidase/nuclease genes from the MSL was associated with a

significant decrease in miltefosine susceptibility, which was restored after re-expression. Metabolomic analysis of parasites lacking the MSL or the two 3'nucleotidase/nuclease genes identified an increase in the parasite's lipid content, including ergosterol; these lipids may contribute to miltefosine resistance by acting as a trap for the drug in the membrane. Parasites lacking the MSL were more resistant to lipid metabolism perturbation caused by miltefosine and the 3'nucleotidase/nucleases are involved in this pathway. Additionally, miltefosine-mediated reactive oxygen species homeostasis was assessed in 26 *L. infantum* clinical isolates, which showed that *L. infantum* parasites lacking the MSL isolated from miltefosine relapsed patients favourably modulated nitric oxide accumulation in host macrophages. Altogether, these data indicate that multifactorial mechanisms are involved in natural resistance to miltefosine in *L. infantum* and that the absence of the 3'nucleotidase/nuclease genes contributes to the miltefosine resistance phenotype.

Keywords MILTEFOSINE RESISTANCE; *Leishmania infantum*; CRISPR-Cas9; 3'NUCLEOTIDASE/NUCLEASE

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P1-086: CHARACTERIZATION OF *Leishmania amazonensis* LINES RESISTANT TO PAROMOMYCIN BY WHOLE GENOME SEQUENCING

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Leishmania amazonensis is responsible for localized cutaneous and diffuse cutaneous leishmaniasis and the second most prevalent species in Brazil. The treatment available is limited to the use of parenteral drugs that are expensive and induce serious side effects, beyond miltefosine that was recently included as an alternative for the treatment of cutaneous leishmaniasis in Brazil. Paromomycin (PM) is an aminoglycoside antibiotic used in the treatment of visceral leishmaniasis intramuscularly and also as topical agent against cutaneous leishmaniasis. Due to the limitations of the treatment and the potential of PM, our aim is to identify potential genes associated with PM susceptibility and resistance in *L. amazonensis*, using clinical isolates with differential susceptibility to PM and PM resistant lines selected *in vitro*. The selection of PM resistant lines was done through three strategies: *in vitro* mutagenesis and stepwise selection in promastigotes and intracellular amastigotes. After confirmation of the resistance phenotype through drug susceptibility assays, we performed whole genome sequence of clinical isolates and PM resistant lines selected *in vitro*. Single nucleotide polymorphisms, mutations, insertions and deletions were identified and potential genes involved in PM resistance are being functionally validated

by gene knockout and/or gene overexpression. *CDPK1*, a gene that codes for a protein kinase previously involved in PM resistance in *L. infantum*, was mutated in 3 of 5 PM resistant lines selected by *in vitro* mutagenesis, but not in clinical isolates that are intrinsically resistant to PM. We inactivated *CDPK1* gene by CRISPR/Cas9 technology and confirmed its role in PM resistance in *L. amazonensis.* L23a is a ribosomal protein involved in translation that interacts with CDPK1 by its phosphorylation, and was previously involved in PM and antimony resistance in *Leishmania* spp. We generated a single-knockout line for this gene in *L. amazonensis* and the transgenic line was resistant only in the promastigote form. Moreover, the PM accumulation in isolates with differential susceptibility was evaluated by fluorescence microscopy and flow cytometry, using a fluorescent analog of PM. We found a direct correlation between PM susceptibility and accumulation of this drug in this species, indicating that a transporter may be involved in the resistance phenotype. This study will contribute for the identification of genes involved in resistance and susceptibility to PM in *Leishmania* that can be potentially useful as markers of resistance in endemic areas where PM is used.

Keywords PAROMOMYCIN; DRUG RESISTANCE; *LEISHMANIA AMAZONENSIS*; WHOLE GENOME SEQUENCING

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P1-087: CONVOLUTIONAL REGRESSION NETWORKS FOR INTRACELLULAR AMASTIGOTE QUANTIFICATION

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A fundamental step for the validation of new drug targets and for drug screening requires quantifying intracellular *Leishmania* parasites (amastigotes) within the infected macrophage. This is traditionally done manually by counting stained parasites under light or fluorescence microscopy, using hundreds of images, a very time consuming process. Recently, alternative automatic counting methods have emerged, based on individual cell segmentation and classification processes. However, these conventional image processing methods present difficulties if there is cell overlap, high confluence, or fuzzy borders. These problems can cause

overestimation or underestimation of the parasite load. In this work we propose a method based on regression convolutional networks (FCRNs) as a first approach to estimate parasite load from fluorescence microscopy images. In this method, the integral of the density maps is related to the number of cells in the image. Density maps are created from the geometric center of each cell, so the problems that occur with segmentation do not occur with this method. For its development, a dataset of 417 images was obtained with two fluorescence channels: CellMask™ Deep Red labelling the macrophage cytoplasm and DAPI for the detection of macrophage nuclei and the parasite kinetoplast (mitochondrial DNA). Images were obtained using murine macrophages infected with *Leishmania major* at different infection multiplicity ratios (5:1, 10:1 and 20:1 parasites:cell) and post-infection times (24 and 120 hours). Individually, 3 experts performed the manual counting of total macrophages, infected macrophages, and number of amastigotes for each of the conditions evaluated, to obtain the parasite load manually and the ground truth (real density map with which the neural network was trained). For the development of the deep learning algorithm, FCRNs were trained to predict density maps related to intracellular parasites, infected macrophages and total macrophages. The number of cells were obtained by integration of the corresponding density map. The parasite load was calculated by determining the mean number of parasites per 100 macrophages in the total number of images analyzed per experiment. The automatic counting produced results similar to the mean of the manual counting (amastigotes R^2 0.97, infected macrophages R^2 0.93 and total macrophages R^2 0.98) with a considerable reduction in time: 6 hours for manual counting of all images per specialist versus 34.2 seconds for counting with the automatic method. Therefore, the proposed method is an efficient alternative for the determination of the parasite load. As an example of use, analyses of macrophage infection with parasite knockouts for genes related to heme metabolism will be shown. These genes are of interest because unlike mammals, *Leishmania* is auxotrophic for heme. Some of these genes were shown to be important for the parasite

intracellular multiplication and the development of the disease in murine models of leishmaniasis.

Keywords NEURAL NETWORKS; INTRACELLULAR PARASITES; AUTOMATIC QUANTIFICATION; HIGH-CONTENT ASSAYS

P1-089: TRANSCRIPTOME ANALYSIS OF THE CLINICAL-IMMUNOPATHOLOGICAL SPECTRUM OF AMERICAN CUTANEOUS LEISHMANIASIS IN AMAZONIAN BRAZIL

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In Brazil, American cutaneous leishmaniasis (ACL) has a broad clinical-immunopathological spectrum resulting from the interaction between different Leishmania species and human immune response. In the Brazilian Amazon, L. (L.) amazonensis and L. (V.) braziliensis represent the species with the highest pathogenic potential, responsible for four clinical forms: localized cutaneous leishmaniasis (LCL/La-LCL/Lb) with balanced immune response and in poles of hyporreactivity and hyperreactivity are diffuse anergic cutaneous leishmaniasis (ADCL/La) and mucosal cutaneous leishmaniasis (MCL/Lb). The aim of this study was to perform transcriptome analysis on the clinico-immunopathological spectrum of ACL in the Brazilian Amazon, using twenty biopsies of skin lesions from individuals with ACL [LCL/La: 4; LCL/L(V.): 6; ADCL/La: 5; MCL/Lb: 5] and six healthy skin samples (control). All of them were submitted to the next-generation RNA sequencing (RNAseq). The transcriptional profile by unsupervised analysis (PCA) revealed 5 clusters: one composed with the control samples, one with the LCL/La, another for LCL/-b, and the two most

distant clusters composed with the polar ADCL/La samples and the polar MCL/Lb ones. A high degree of disturbance in gene expression was detected in skin biopsies infected with Leishmania in comparison to healthy controls. To determine differentially expressed genes (DEGs) the edgeR tool was used based on two criteria (log2 fold-change > 1 and < -1; Padjusted ≤0.05), revealing up- and down-regulated genes, respectively: ADCL/La (2,249-1660), LCL/La (2185-2270), LCL/L(V) (2171-1934), and MCL/Lb (2380-1994). The heatmap of all DEGs concerning to the four clinical forms did not show clear distinct patterns among groups, but some contrasting gene blocks were observed especially between the polar forms (ADCL and MCL). Through the enrichment analysis of biological pathways using the GSEA tool filtered by NES values greater than 1 or less than -1 and Padjusted ≤ 0.05, and with the level 3 Reactome database, shared pathways at the same level of expression were observed and others with distinct level of activation among the four clinical groups. Upregulated pathways (NES>1) related to the immune system in general, such as interferon signaling, MHC class II antigen presentation, toll-like receptors cascades, neutrophil degranulation, were shown to be activated in similar fashion among groups. The pathways related to metabolism and cell cycle showed reduced activity (NES <-1) and were composed with downregulated genes. However, the class I MHC mediated antigen processing and presentation, as well as the DDX58/IFIH1-mediated induction of interferon-alpha/beta pathways were upregulated in MCL cases and downregulated in ADCL. These findings reveal the need to deep the analysis at the level of genes exclusively expressed in each group, which may lead to the identification of gene groups and pathways that could support the understanding of components of the immune response involved in resistance or susceptibility to the disease, as well as to characterize genetic markers associated with different clinical outcomes in ACL.

Keywords AMERICAN CUTANEOUS LEISHMANIASIS; *L. (L.) amazonensis*, *L. (V.) braziliensis*; TRANSCRIPTOME ANALYSIS

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P1-090: ENDOPLASMIC STRESS AFFECTS THE COINFECTION OF *LEISHMANIA AMAZONENSIS* AND THE *PHLEBOVIRUS (BUNYAVIRIDAE) ICOARACI*

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Phlebotominae sand flies vectorize *Leishmania* parasites and can also transmit to vertebrates some arboviruses belonging to the genus Phlebovirus. The geographical overlap of Leishmaniasis and phleboviruses transmission and finding co-infected sand flies suggests the potential public health relevance of such co-infections in humans. In previous work, we showed that the co-infection of Leishmania amazonensis with the Icoaraci virus (ICOV), an Amazonian phlebovirus isolated from natural reservoirs of Leishmania amazonensis enhances the infection of the parasite both in vivo and in vitro. Our recent reports demonstrated that *Leishmania* infected macrophages exhibit the Endoplasmic reticulum stress response, which is required for parasite replication. Herein we demonstrate that Icoaraci phlebovirus enhances and sustains the IRE1a and PERK endoplasmic reticulum stress pathways in the co-infection model. We demonstrated the relative increase of the XBP1 spliced product and the increased expression of genes related to phospholipid biosynthesis under the IRE1/XBP1 pathway regulation. The activation of PERK/ATF4, another ER stress branch, was also observed in cells infected with ICOV and in co-infected macrophages. Importantly, we observed the increased expression of anti-oxidative responsive genes during co-infection. The sustained and enhanced activation of the ER stress pathways elucidates a possible mechanism to justify the favoring of infection by *L.amazonensis* in a model of co-infection with the ICOV and open the possibility of investigating the importance of these pathways in the aggravation of the infection by other Leishmania species as well as co-infection with other phleboviruses.

Keywords PHLEBOVIRUS; ICOARACI; ER STRESS; *Leishmania*

Financing CNPq, CAPES AND FAPERJ

P1-091: COMBINATORIAL APPROACHES TO SELECT HIGH-AFFINITY BINDERS OF EXPOSED MEMBRANE PROTEINS FROM *LEISHMANIA BRAZILIENSIS*

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American tegumentary leishmaniasis (ATL) affects the skin and/or mucosa and has a significant public health impact in endemic regions across the Americas. *Leishmania braziliensis* is the main causative agent in the Americas. Accurate diagnosis of ATL requires a combination of diagnostic tests. Molecular techniques are superior to the conventional methods such as microscopy or culture, particularly for lesions with low parasite loads. Despite simplification and robustness of modern DNA detection workflows, they still need highly trained personnel and specialized infrastructure that are rarely available in rural endemic areas. Therefore, accessible and affordable alternative diagnostic methods are needed. A method for parasite cell enrichment during sample preparation for diagnostics could boost the sensitivity and specificity of diagnostic tools for ATL. Here, we use two combinatorial techniques to develop high affinity molecules aiming to capture *L. braziliensis* cells. Two peptides, designated P2 and P3, derived from extramembrane domains of two membrane proteins of *L. braziliensis*, were selected as capture targets by bioinformatics and ribosome profiling data analysis. The predicted structural models confirmed that the selected peptides are exposed at outer membrane regions. Both peptides were synthesized chemically with a covalently linked biotin and used as target molecules for SELEX (Systematic Evolution of Ligands by EXponential

enrichment) and Phage-Display. Five candidates of each, DNA and peptide aptamers, for each membrane protein target were identified by sequencing and bioinformatics analysis. To understand the interactions between the candidate aptamers and the target membrane peptides, homology models of the candidate aptamers and membrane proteins were constructed followed by molecular docking analysis. The binding affinity of the aptamer candidates is currently being investigated by ELISA or ELONA (enzyme-linked oligonucleotide assay) for the peptide or DNA aptamers, respectively. Additionally, their capacity to bind the membrane proteins will be tested using *L. braziliensis* promastigote protein extracts. Preliminary results with ELONA confirmed a set of promising DNA aptamers against P2. Altogether, the aptamers developed in this work will be immobilized in magnetic beads to develop a tool aiming to capture and concentrate *Leishmania* cells from different types of samples, likely increasing the sensitivity of downstream molecular detection workflows.

Keywords APTAMERS; SELEX; PEPTIDES; PHAGE-DISPLAY; *Leishmania braziliensis.*

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P1-092: COMPARATIVE ANALYSIS OF PROTEIN PARTNERS CO-PRECIPITATED WITH THE THREE DIFFERENT *LEISHMANIA* PABP HOMOLOGUES

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Cytoplasmic Poly-A Binding Proteins (PABPs) are unique and conserved RNA binding proteins (RBPs) that bind specifically to the poly-A tails of nearly all eukaryotic mRNAs. These proteins are known to be involved in most processes associated with the metabolism of mRNAs, many of which can be regulated. Multiple PABP homologues can be found within different organisms, with functional distinctions generally not well defined. In *Leishmania*, where the regulation of mRNA metabolism and translation is preponderant for gene expression regulation, three PABP homologues were identified which are constitutively expressed as abundant cytoplasmic proteins. PABP2 and PABP3 migrate to the nucleus upon inhibition of transcription, co-precipitate together and bind to similar mRNA populations. These are different from those bound by PABP1, recently shown to specifically bind to ribosomal protein mRNAs. *Leishmania* PABP1 has also been shown to bind specifically to the RNA binding protein RBP23 and selected translation initiation factors (EIF4E4, EIF4G3), suggesting a specific role in regulating mRNA translation. In order to contribute further to the understanding of the different roles of the *Leishmania* PABPs, here we sought to define proteins specifically associated with PABP2 and PABP3 and compare them with those co-precipitated with PABP1. Assays were carried out under similar conditions using C-terminally HA-tagged proteins expressed in the *Leishmania infantum* promastigote stage in exponentially

grown cells. The tagged proteins were used in immunoprecipitation reactions followed by the identification of co-precipitated protein partners through mass spectrometry. Our results reveal a much larger set of proteins co-precipitated with PABP2 than with PABP3 or PABP1. Nevertheless, and as expected, many protein partners were found with both PABP2 and PABP3, with these two PABP homologues also co-precipitating efficiently with each other. Top-most partners co-precipitated with both, and not generally found with PABP1, include several RRM containing RBPs (DRBD2, RBP12 and the mitochondrial RBP1 and 2) and the translation factor EIF4G5. Proteins specifically co-precipitated with PABP2 include a nuclear export factor (XPO1) and the La RBP. PABP3 also co-precipitates with RBP23 and EIF4E4, seen with PABP1, but no clear PABP3 specific protein partner was identified. Relevant proteins associated with all three PABP homologues include the Zinc-Finger ZC3H41, the hypothetical LINF_050009500, the ALBA RBPs and the cell-cycle regulated kinase CRK3. Interestingly, PABP1 and the PABP2/PABP3 pair associate strongly and alternatively with two distinct uncharacterized proteins having a NTF2/Ras-GAP domain and whose orthologues in *Trypanosoma brucei* are cytoplasmic proteins identified as post-transcriptional activators of gene expression. The data suggest that these uncharacterized proteins may have specific and unknown roles associated with PABP function in *Leishmania*. Other relevant interactions identified also need to be investigated further in order to better define how the three PABPs function in different processes affecting the mRNA metabolism in *Leishmania* and related parasites.

Keywords RNA BINDING PROTEIN, PROTEIN SYNTHESIS, GENE EXPRESSION REGULATION, TRANSLATION INITIATION FACTOR, eIF4F

P1-094: EVALUATION OF THE EIF4E3 TRANSLATION INITIATION FACTOR FROM DIFFERENT *LEISHMANIA* SPECIES

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In eukaryotes, recognition of mRNAs by eIF4E is a key step during the initiation stage of the mRNA translation. eIF4E is a subunit of the eIF4F complex (with eIF4AI and eIF4G) and is responsible for promoting the recruitment of ribosomal subunits to the mRNA. *Leishmania* species and other protozoan parasites from the Trypanosomatidae family are characterized by unique features associated with translation initiation and which include the presence of six eIF4Es. It is known that EIF4E3 is a phosphoprotein, but the phosphorylation sites may vary between different species. An example is the phosphorylation at serine 75, present only in *Leishmania amazonensis* and *Leishmania mexicana*. EIF4E3 together with EIF4G4 is part of one of the eIF4F-like complexes essential for cell survival, but the main partners of this complex and the role of phosphorylation in translation are still unclear. Here we aimed to comparatively evaluate the native EIF4E3 from *Leishmania infantum* (LiEIF4E3) and *L. amazonensis* (LaEIF4E3), as well as a LaEIF4E3 variant mutated at the specific serine 75 residue (LaEIF4E3S75A). First, to evaluate their expression profile, growth curves of transgenic cell lines expressing HA-tagged versions of LiEIF4E3, LaEIF4E3 and LaEIF4E3S75A were used. LiEIF4E3 and LaEIF4E3 showed different electrophoretic migration profiles, with a major isoform seen for LiEIF4E3 which did not vary during promastigote cell growth. Meanwhile, LaEIF4E3 was associated with multiple isoforms, the higher molecular weight ones associated with the stationary growth phase, and the lower molecular weight isoforms present only during logarithmic growth,

suggesting species-specific and growth-phase specific post-translational modifications. The LaE3S75A variant showed a profile similar to the LiEIF4E3 with the disappearance of the heavier isoforms. Soluble cytoplasmic extracts were then produced followed by their use in immunoprecipitation reactions which were sent for analyses by mass spectrometry for the identification of co-precipitated protein partners. The LiEIF4E3 mass spectrometry data revealed its co-precipitation with its known EIF4G4 partner as well as subunits of the eIF3 translation complex and ribosomal proteins. EIF4AI, a RNA helicase and eIF4A homologue, was not found, but two homologues of the related Ded1p RNA helicase (DED1 and HEL67) were detected. In yeast Ded1p act together with the eIF4F complex stimulating translation, and their identification as EIF4E3 partners in *Leishmania*, suggests yet undefined specific aspects of its mode of action. Other co-precipitated proteins include selected RNA binding proteins and the protein kinase RDK2, candidate for mediating EIF4E3 phosphorylation. Unexpectedly, the native LaEIF4E3 did not co-precipitate with most translation-related proteins, but the LaE4S75A, co-precipitated with a protein profile similar to LiEIF4E3. The results indicate specific regulatory events targeting EIF4E3 in different *Leishmania* species, with the phosphorylation in *L. amazonensis* possibly acting by inhibiting translation. They also reinforce the likely but mostly unknown roles for different helicases during mRNA translation in *Leishmania* and other eukaryotes.

Keywords RNA BINDING PROTEIN; PROTEIN SYNTHESIS; GENE EXPRESSION REGULATION; TRANSLATION INITIATION FACTOR

P1-096: USING ECTOPIC LENTIVIRAL EXPRESSION TO DECIPHER THE ROLE OF *LEISHMANIA* CASEIN KINASE 1 IN THE MACROPHAGE

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To efficiently ensure its survival and proliferation, the *Leishmania* parasite manipulates its host cell via the secretion of parasite effector proteins, such as *Leishmania* casein kinase 1 paralog 2 (L-CK1.2). We showed that L-CK1.2 is closely related to human CK1, essential for intracellular parasite survival. Furthermore, several evidences suggest that L-CK1.2 has been evolutionary selected to interact with and to phosphorylate host proteins. We showed *in vitro* that L-CK1.2 regulates specific pathways in the macrophage host cell, such as trafficking, apoptosis, actin organisation and translation, all of which are known to be modulated during *Leishmania* infection, thus revealing that L-CK1.2 is a key player in host-parasite interactions. To confirm these data and study the impact of this kinase on the macrophage, without the interference from other secreted parasitic proteins, we generated two cell lines of immortalized J774 macrophages expressing only *Leishmania donovani* CK1.2 tagged with V5 (LdCK1.2-V5) in a non-inducible and inducible manner, as well as the two corresponding control cell lines expressing ZsGreen. To do so, we generated two lentiviral expression vectors by cloning LdCK1.2 to obtain pFuGw-LdCK1.2-V5-His6-IRES-Neo for constitutive expression and FUW-tetO-MCS-LdCK1.2-V5-His6-Neo for inducible expression of LdCK1.2-V5. These plasmids and the corresponding controls expressing ZsGreen were transduced in J774 macrophage cell line. These four cell lines were validated by microscopy and Western Blot

analysis. Next, we optimized the induction conditions on the control cell line expressing ZsGreen under an inducible promoter by testing different concentrations of doxycycline and different induction times to allow its maximum expression, while avoiding a toxic effect on the macrophage. The optimal expression was obtained on day 3 of induction at 2 µg/mL of doxycycline or on day 6 at 0.5 µg/mL. The same strategy was applied to the cell line expressing *Leishmania* CK1.2-V5 under an inducible promoter. Apart from a slower growth, we did not notice any phenotype on non-polarized macrophages. We are currently establishing the localization of L-CK1.2-V5 in the macrophage by immunofluorescence, using the constitutively expressed CK1.2-V5 cell line. Our first observations suggest a cytoplasmic and peri-nuclear localization of the *Leishmania* kinase in the macrophage, localization similar to that described for mammalian CK1s. We are also performing phospho-proteomic analyses to determine whether ectopic L-CK1.2 modifies the phospho-proteome of the transgenic macrophage and to identify its potential targets. These substrates will be compared to those identified *in vitro*. In conclusion, we have established a cellular model that will allow us to highlight L-CK1.2 host cell functions and to identify potential targets for host-directed therapies.

Keywords CASEIN KINASE 1; HOST-*Leishmania* INTERACTIONS; EXTRACELLULAR VESICLES; PHOSPHO-PROTEOMICS

P1-097: COMPARATIVE EVALUATION OF THE INTERACTIONS OF THE EIF4G3 AND EIF4G4 TRANSLATION INITIATION FACTORS WITH PARTNER PROTEINS IN *Leishmania infantum*

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Leishmania and related kinetoplastid protozoa are characterized by atypical molecular processes associated with their gene expression, such as polycistronic transcription and regulation of gene expression mediated mainly by post-transcriptional mechanisms. Many of these mechanisms likely target events associated with the metabolism of mRNAs, such as during their translation, but not many examples are well understood. In eukaryotes, translation of mRNAs begins with the binding of the eIF4F complex (based on the eIF4E, eIF4A and eIF4G subunits) to the cap nucleotide found at the 5' ends of the mRNAs. This is a well-regulated event in different organisms that is required for the efficient recognition of the mRNAs by the ribosomes. The eIF4G subunits is a large modular protein with multiple domains and binding motifs, but which has only been better characterized from a few selected organisms. Five eIF4G homologues have been reported conserved in all kinetoplastid species investigated. Two of those, EIF4G3 and EIF4G4, have been implicated as having direct roles during mRNA translation, but these roles need to be better defined. To enhance our understanding of their mode of action, this work sought to do an extensive comparative analysis investigating protein partners associated with each of the two eIF4G homologues. Two transgenic *Leishmania infantum* cell lines were first generated expressing each of the two proteins, tagged with a C-terminal HA epitope. Both were expressed in the promastigote life stage as more than one isoform, with EIF4G4 being represented by two distinct bands only at the stationary phase of growth.

Whole cytoplasm extracts were then prepared from the two cell lines under mild, detergent-free, conditions, in order to avoid disrupting protein complexes. The HA-tagged proteins were then immunoprecipitated with anti-HA beads and co-precipitated protein partners identified through mass spectrometry. A first assessment of the data revealed the expected EIF4G3 and EIF4G4 binding to EIF4AI, the *Leishmania* eIF4A homologue, and known eIF4E partners, with EIF4E4 co-precipitating with EIF4G3 and EIF4E3 with EIF4E4. A large number of ribosomal proteins and various translation factors co-precipitated with both tagged baits, confirming their involvement in translation. A restricted set of RNA binding proteins and RNA helicases were also found with both sets of immunoprecipitated samples, including ZC3H41, RBP43, PUF2, HEL67 and Alba domain proteins. EIF4G3, but not EIF4G4, was also found associated with several tRNA synthetases as well as the eRF3 factor, required for translation termination. Specific proteins co-precipitated with EIF4G3 also include a large number of enzymes, heat-shock and chaperone proteins and the RNA binding protein RBP23. In contrast, EIF4G4 was found to be enriched with a distinct set of chaperones as well as several specific RNA binding proteins, small GTP binding proteins and other factors involved with RNA processing. These results are consistent with the two eIF4G homologues having related but distinct functions during translation and its regulation and possibly linked to distinct mRNA targets. Further work will be required to investigate direct interactions with distinct protein partners as well as the requirements within each eIF4G homologue for their adequate activity.

Keywords TRANSLATION INITIATION FACTOR; eIF4F; RNA BINDING PROTEIN; PROTEIN SYNTHESIS; GENE EXPRESSION REGULATION

P1-098: EVALUATION OF MACROPHAGE IN VITRO MIXED INFECTION BY TWO *LEISHMANIA (VIANNIA) SPECIES*

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Mixed infections are frequent events that occur naturally in several infectious diseases. In leishmaniasis, mixed infections by different species or strains of *Leishmania* parasites have already been identified in the insect vector and in different mammalian hosts, including man. Mixed infections have been related to disease severity, failure in therapeutic response and parasitic persistence. There may be an interaction between species and therefore an inhibitory or stimulatory effect during a mixed infection. Considering the *Leishmania*-macrophage interaction, important differences can be observed when comparing single *Leishmania* species infection and those provoked by two or more species, influencing the host immune response. In this work we aim to investigate experimental *in vitro* macrophage mixed infections by *Leishmania* species associated with distinct clinical courses: *Leishmania (Viannia) naiffi* (Ln_IOC/L3310), associated with self-healing localized cutaneous leishmaniasis (LCL) and *L. (V.) braziliensis* (Lb_IOC/L3356), associated with non-self-healing LCL. Single infection assays using Lb_IOC/L3356 and Ln_IOC/L3310 and mixed infection assays with the same strains were performed in J774 macrophage lineage followed up to 72h. MOI of 10:1 and 20:1 parasite/macrophage were evaluated. The percentual of infected macrophages was higher when mixed infections (Lb_IOC/L3356+Ln_IOC/L3310) were performed comparing to single infections with Lb_IOC/L3356, but no differences were observed when comparing to Ln_IOC/L3310 infections. Regarding the number of amastigotes/infected macrophages, the mixed infection group (MOI

10Lb_IOC/L3356 + 10Ln_IOC/L3310 : 1) had a higher number of parasites in relation to the group infected with *L naiffi* but not in relation to the group infected with *L. braziliensis.* Infections using MOI 5Lb_IOC/L3356 + 5Ln_IOC/L3310 : 1 did not show differences in the number of amastigotes/macrophage when comparing to single infections by both species. qPCR was employed to identify the number of parasites representing each species during mixed infections. It was observed that in 24 hours the mixed infection (Lb_IOC/L3356+Ln_IOC/L3310) presents higher number of Lb_IOC/L3356 compared to the group infected with Lb_IOC/L3356 only, but no significant differences were observed in the number of Ln_IOC/L3310 in mixed infection comparing to single infections by this species. After 72h, the number of Ln_IOC/L3310 in the mixed infected cultures was lower than the number of Ln_IOC/L3310 in the single infection with this species. However, the proportion of each species in mixed infections at that time point of infection were similar. Differences were observed accordingly to the MOI employed for macrophages infections either in single or mixed infections. The results obtained so far point to favoring the establishment of infection when macrophages are submitted to the infection of both species since the beginning. We are now evaluating immunological response of macrophages during single and mixed infections measuring cytokines.

Keywords MIXED INFECTION; *Leishmania* (*Viannia*) *naiffi*; *Leishmania* (*Viannia*) *braziliensis*

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P1-099: CHANGES IN TWO-DIMENSIONAL GEL ELECTROPHORESIS PATTERNS OF RIBOSOMAL FRACTION IN AN AXENIC DIFFERENTIATION MODEL OF QUIESCENCE IN *Leshmania mexicana*

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Leishmaniasis is a tropical disease caused by the *Leishmania* parasite. It has two stages: promastigote and amastigote, the latter being the cause of disease in the mammalian host. In *Leishmania*, a state of quiescence has been described in the amastigote. Quiescence is clinically relevant because it allows pathogens to persist in the host and reactivate disease years after clinical cure. It is important to understand the mechanisms that trigger quiescence to propose strategies for the control of the disease. Leishmania gene expression is noteworthy because genes are constitutively expressed with only 0.2% to 5% of different mRNA sequences between stages. Therefore, the regulation of gene expression must occur mainly at the post-transcriptional level. We hypothesize that ribosomes may play a discriminatory role to translate specific mRNAs. Recent studies suggest that the heterogeneity of ribosomal components, i.e. rRNA and ribosomal proteins, could play a role in the regulation of gene expression by selective association with different mRNA subgroups and/or performing extra-ribosomal functions. In yeast, the differential expression of paralogous ribosomal proteins was associated with the transition from a proliferation stage to quiescence. Therefore, we propose that the ribosomal fraction of quiescent amastigotes must present significant proteomic changes. To obtain a population of mainly quiescent cells, amastigotes were cultured in conditions of nutrient-poor medium and hypoxia. Using flow cytometry, the quiescence state was monitored by analyzing a biosensor consisting of the insertion of GFP in the 18S ribosomal DNA locus. The decrease in 18S

ribosomal RNA is a feature of quiescence found in other organisms and previously reported in *Leishmania*. We showed that the conditions of nutrients limitation and hypoxia allow us to obtain a quiescent population with 90.13% of cells that are rGFP negative. This group was compared with stationary promastigotes and the previous model of axenic amastigotes, which presents a heterogeneous population with 47.15% of rGFP negative. The ribosomal fraction from the group with high percentage of quiescent amastigotes was compared with stationary promastigotes and analyzed by two-dimensional gels electrophoresis, using silver stain. Comparison of two-dimensional gel electrophoresis patterns using Melanie 2D gel analysis software showed that 16 out of 220 corresponding individual spots presented a differential pattern. By comparing the isoelectric points and molecular weights of each spot with Uniprot database of ribosomal proteins of *Leishmania mexicana*; we were able to propose that two of them may be ribosomal proteins: RPL7/RPL12-like, which was underexpressed, and RPS4, which was overexpressed. Interestingly, the ribosomal protein RPL7/RPL12 decrease was reported in bacteria to be associated with low efficiency in protein production, a characteristic of the quiescent state. From the results mentioned above, we conclude that it is important to identify the translational machinery components or other proteins present in the ribosomal fraction by mass spectrometry to look for differences that explain the phenotype of *Leishmania* quiescent amastigotes.

Keywords *Leishmania*; AMASTIGOTE, QUIESCENCE; TWO-DIMENSIONAL GEL; RIBOSOMAL PROTEINS

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P1-100: TRANSCRIPTIONAL PROFILE OF REACTIVE OXYGEN AND NITROGEN SPECIES PRODUCTION BY PHAGOCYTIC CELLS IN HUMAN *Leishmania infantum* INFECTION

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The first line of defense against infectious agents involves an active recruitment of neutrophils and monocytes/macrophages to the infection site. These cells are able to engulf invading microbes into a phagosome which are exposed to numerous microbicidal events that include acidification and the production of reactive oxygen and nitrogen species (ROS and RNS) by the specialized enzymes NADPH oxidase complex and inducible nitric oxide synthase (iNOS). The production of these leishmanicidal effector molecules (ROS/RNS) is a key event for the clinical outcome of *Leishmania infantum* infection. Worth mentioning, nitric oxide (NO), primary source of all RNS in biological systems, is of pivotal importance to control murine leishmaniasis, while the leishmanicidal effects of ROS seem to be greater in human infection. To better understand the transcriptional status of pathways and genes related to ROS and RNS production by phagocytes, we accomplished a next-generation RNA sequencing (RNAseq) in whole blood of individuals from an endemic Brazilian Amazon region (Pará state, Brazil) for visceral leishmaniasis (VL), including active VL patients infected with *Leishmania (L) infantum chagasi*

(VL, n=10) and asymptomatic individuals (AI, n= 15) presenting high response to the intradermal leishmanin test and low production (less than the cut-off titer 80) of specific antibodies. The mRNA expression of both groups was compared to that found in healthy endemic controls (n=11). The pathway "ROS and RNS production by phagocytes" (Reactome database) was significantly upregulated only in AI (NES AI= 1.9, Padjusted ≤ 0.001; NES VL= - 0.9, Padj ≥ 0.05). Exploring the genes of this pathway, we observed an overexpression (Padj ≤ 0.01) of those encoding components of vacuolar ATPase (V-ATPase) - a multisubunit enzyme that mediates acidification of intracellular compartments in phagocytes - only in AI, as well as the SLC11A1 gene (Padj ≤ 0.01) that pumps out iron from phagolysosomal milieu to cell cytoplasm, which effectively inhibits *Leishmania* growth and survival in human macrophages. In contrast, NCF4 gene, which encodes the subunit $p40^{phox}$ of the NADPH-oxidase complex, a crucial enzyme responsible for generating ROS, was downregulated only in VL (Padj ≤ 0.01). mRNA expression of NOS2 that encodes iNOS to produce NO was not observed in both AI and VL groups. Relative to phagocytes, the pathways "enriched in neutrophils and "enriched in monocytes" (Blood Transcriptional Modules) were both significantly upregulated in AI (NES= 2.8 and 3.0, respectively), opposed to the active VL patients (NES= - 2.4 and – 2.1, respectively). Besides, the proportion of macrophages and neutrophils were higher in AI than in VL group (Deconvolution Tool). In summary, the transcriptional profile of asymptomatic infection was characterized by upregulated genes that are evolved in ROS production by phagocytes, acidification and low availability of iron in phagolysosomes, all of them related to *Leishmania* restrain. VL patients showed an opposite profile, besides a downregulation of $p40^{phox}$, which could impair the assembly of the NADPH oxidase complex and ROS production. Finally, blood transcriptomics showed a prominent role of ROS for controlling intracellular survival of parasites in human *Leishmania (L) infantum chagasi* infection, in contrast to NO.

Keywords BLOOD TRANSCRIPTOME; HUMAN *Leishmania infantum* INFECTION; ROS AND NO PRODUCTION; PHAGOCYTES

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P1-101: MOLECULAR SIGNATURE OF CD8⁺ T-CELL EXHAUSTION IN HUMAN VISCERAL LEISHMANIASIS

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CD8⁺ T-cells have been shown to contribute to *Leishmania* sp. control. However, persistent infections, as it occurs in visceral leishmaniasis (VL), may cause dysfunctional CD8⁺ T-cell response, which has implications for pathogen survival and replication. Studies on exhaustion process of CD8⁺ T-cells in VL are mainly based on experimental models, but those addressing human VL have been few and limited. Therefore, to better understand the incidence of exhausted CD8⁺ T-cells in human active VL, we investigated the gene expression of inhibitory receptors that are considered markers of cellular exhaustion processes. In that order, we analyzed the transcriptional profiling (RNA seq) of peripheral blood CD8⁺ T-cells of three groups from a VL endemic area in the Brazilian Amazon region composed by active VL patients infected with *Leishmania (L.) infantum chagasi* (n=10), asymptomatic individuals (n= 15) with high response to specific leishmanin skin test [*L. (L.) infantum chagasi* promastigote antigen] and low production (less than the cut-off titer 80) of specific antibodies, and compared both with CD8⁺ T-cells profile of healthy individuals from endemic area (control group n=11) focusing the expression of genes encoding the cytotoxic T

lymphocytes antigen 4 (**CTLA-4**), programmed cell death-1 (**PD-1**), lymphocyte activation gene 3 (**LAG-3**) and T-cell immunoglobulin and mucin-domain containing-3 (**TIM-3**). In addition, we have also measured the parasite load by q-PCR. Herein, 100% of VL patients have showed a marked upregulation ($P_{adjusted} \leq 0.05$) of all the inhibitory $CD8^+$ T-cells receptors (PD-1 Log2FC= 2.48, LAG-3 Log2FC= 2.2, CTLA-4 Log2FC = 1.35, TIM-3 Log2FC= 1.09), in contrast to the asymptomatic individuals who have not changed their gene expressions ($P_{adjusted} > 0.05$) (PD-1 Log2FC= 0.09, LAG-3 Log2FC= - 0.13, CTLA-4 Log2FC = -0.01, TIM-3 Log2FC= 0.12). Respective to the parasite load, it was at least 50-fold higher in VL patients than in asymptomatic individuals. In summary, we have identified a number of differentially expressed inhibitory genes in circulating $CD8^+$ T-cells from VL patients, along with high parasite load. Finally, these data sets point out that $CD8^+$ T-cells are driven to exhaustion in human active VL, impairing their ability to contribute to the protective cellular immune responses and parasite restrain.

Keywords HUMAN VISCERAL LEISHMANIASIS; *Leishmania infantum;* INHIBITORY RECEPTORS; $CD8^+$ T-CELL EXHAUSTION

Financing grant #2014/50315-0 FAPESP, Instituto Evandro Chagas (Brasil), UFPA (Brasil), LIM50 HC-FMUSP.

P1-102: CHARACTHERIZATION OF MILTEFOSINE TRANSPORTER GENE OF *Leishmania braziliensis*

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Around 20 species of *Leishmania* are responsible for leishmaniasis and at least 8 of them are endemic in Brazil. Cutaneous leishmaniasis in Brazil is caused mainly by *L. braziliensis* that is transmitted mainly by vectors belonging to the genus *Lutzomyia*, the most diverse and with a vast territorial distribution in South America. There are no vaccines for leishmaniasis and the treatment has a limited number of drugs that are toxic and induce several side effects. Miltefosine is an alternative drug that was recently approved for the treatment of cutaneous leishmaniasis in Brazil. Recent studies have shown a significant variation in *in vitro* susceptibility to miltefosine in *L. braziliensis* isolates that were never exposed to miltefosine, suggesting that intrinsic resistance may occur in this species. The uptake of miltefosine in *Leishmania* is mainly performed by the miltefosine transporter (MT), a protein that belongs to the P-type ATPase family, in association with its subunit Ros3 and that are located in plasma membrane of the parasite. In this study, our aim is to understand the role of MT in miltefosine susceptibility in *L. braziliensis* through strategies like overexpression and knockout by CRISPR/Cas9 technology. We also performed whole genome sequencing of *L. braziliensis* (M2903 strain) and our previous analysis indicated that there are three copies of *MT* gene, differently for what is available at TritrypDB, where only two copies are

annotated. Sequence analyses of these three copies indicated some non-synonymous single nucleotide polymorphisms (SNPs), but it still is unknown if these changes may affect miltefosine susceptibility in this species. The episomal overexpression of one of these *MT* gene copies in a heterologous species, *L. amazonensis*, indicated that this gene is functional and parasites were more susceptible to miltefosine than parasites transfected with the empty vector. Furthermore, we have already generated a *L. braziliensis* line with at least two copies of *MT* gene inactivated by CRISPR/Cas9. Miltefosine susceptibility assays with this transgenic line is in progress. Our findings will be helpful to predict whether MT may also affect the effectiveness of this drug against this species, and whether treatment with alternative drugs is need when MT is not functional, for example.

Keywords *Leishmania braziliensis*; MILTEFOSINE; MILTEFOSINE TRANSPORTER.

Financing FAPESP

P1-102: CHARACTERIZATION OF ZC3H41, A ZINC FINGER CCCH-TYPE PROTEIN PARTNER OF POLY(A) BINDING PROTEIN (PABP) HOMOLOGUES FROM *Leishmania infantum*

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Leishmania and related protozoans are characterized by unique genetic processes which include the regulation of their gene expression being mediated mainly by post-transcriptional mechanisms. These have as their main targets the mRNAs, whose processing, transport, stability and translation may all be regulated. All these events generally require the action of multiple RNA binding proteins (RBPs), many of which capable of interacting with regulatory motifs on mature mRNAs. Other RBPs may have more general roles associated with the mRNA metabolism, such as the cytoplasmic poly-A binding proteins (PABPs), RBPs that interact with the poly-A tail at the 3' end of the mRNAs and are involved in many steps of their metabolism, most prominently translation. RBPs are generally classified according to the types of structural domains involved in RNA-binding such as the RRM domain (found within the PABPs) and the CCCH zinc finger (ZF) motif. The CCCH-type proteins are usually related to mRNA decay processes and their regulation, however some are also involved in translation. A complete set of 131 *T. brucei* and 120 *L. major* proteins were identified having between one and five putative CCCH-ZF motifs, with several in *T. brucei* found to have roles related to mRNA metabolism and cell survival. In this study, we focused on the study of the *Leishmania infantum* ZC3H41, characterized by the presence of a single CCCH-ZF motif followed by KH and helicase domains. ZC3H41 was previously reported to associate specifically with the Spliced-Leader (SL) sequence, found on the 5' end of all *Leishmania*

mRNAs. It was also identified by us as one of the top-most proteins co-precipitated with the three *Leishmania* PABPs. Here, homology analysis revealed ZC3H41 to be found throughout all trypanosomatids and kinetoplastids investigated, but with no clear homologues found in organisms outside the Euglenozoa phylum and lacking the SL sequence. ZC3H41 was found to be expressed constitutively in *Leishmania* promastigotes as a highly abundant protein found to be expressed in levels similar to those seen for the PABP homologues. Immunoprecipitation (IP) assays followed by mass spectrometry identification of co-precipitated partners identified an uncharacterized protein (LINF_050009500), previously found substantially enriched with all three *Leishmania* PABPs and the SL RNA, as the most likely ZC3H41 partner. Similar IP assays were performed followed by RNAseq analyses to define mRNA targets, revealing a non-specific profile of bound mRNAs including transcripts co-precipitated with the three PABPs. Phyre2 modelling using the LINF_050009500 sequence predicted a SKP1-like structure, found in proteins associated with cell-cycle regulation and selective, proteasome mediated, protein degradation. Phylogenetic analysis identified LINF_050009500 orthologues in most but not all kinetoplastids investigated, revealing a more restricted distribution than ZC3H41. Current studies are evaluating a possible direct interaction between ZC3H41 and LINF_050009500 in order to better define how the two proteins may be involved in novel cellular processes possibly linking mRNA regulation with protein degradation.

Keywords TRYPANOSOMATIDAE; ZC3H41; PABP1; SKP1

P1-104: TRANSLATIONAL REPROGRAMMING AS CENTRAL DRIVER OF ANTIMONY-DRUG RESISTANCE IN *LEISHMANIA* PARASITES.

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Drug resistance is a major mitigating factor for leishmaniasis treatment, resulting in reduced therapeutic efficacy of pentavalent antimonials, the main antileishmanial drug. Nevertheless, the molecular mechanisms modulating the antimony-resistant phenotypes remain poorly understood. Since *Leishmania* parasites have a limited use of transcriptional control, post-transcriptional mechanisms take a prominent role in the coordination of gene expression. Here, we used a translatomic approach, coupling polysome profiling and deep RNA-sequencing to determine if the resistance to antimony is modulated at the translational level. The translatome of the antimony-resistant strain was dramatically different from the sensitive strain even in the absence of the drug and included 2431 differentially translated transcripts. Our data support that during development of drug resistance remodeling of translation serves as a preemptive adaptation to efficiently compensate the loss of biological fitness once they are exposed to the antimony. The translatome data were partially validated by RT-qPCR and proteomic analyses, identifying not only previously reported antimony-resistance markers, but also several potentially new ones. In contrast, drug-resistant parasites exposed to antimony drug activated a highly selective translation of only 156 transcripts involved in interconnected biological processes, such as improved energy metabolism and oxidative response, drug inactivation, surface protein remodeling, and drug efflux. Thus, antimony-resistant parasites display a complex, preemptive adaptation to

the drug through global translatome remodeling in comparison with sensitive parasites, allowing later for a highly targeted and coordinated response to drug challenge. Classification of gene variants and differentially translated transcripts highlight importance of mRNA translation components, surface protein rearrangement, optimized energy metabolism, and improved antioxidant response. We propose a novel model which establishes translational control as a major driver of antimony-resistant phenotypes.

Keywords LEISHMANIASIS; ANTIMONY RESISTANCE; DRUG CHALLENGE; TRANSLATOME ANALYSIS; TRANSLATIONAL MASTER REGULATORS

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P1-105: GENOTYPIC CHARACTERIZATION OF *Leishmania* (*Leishmania*) *major*-LIKE ISOLATES IN BRAZIL

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American cutaneous leishmaniasis (ACL) is considered a zoonosis, maintained in nature by synanthropic animals, with the secondary participation of domestic animals and accidentally man. Currently, ACL is one of the most important endemic diseases for public health in Brazil, with social and economic repercussions. In Brazil, eight species of the genus *Leishmania* have previously been characterized as causing ACL, including *L. major*-like isolates. *L. major*-like strains have been isolated in several countries in Latin America (Brazil, Ecuador, Paraguay, Venezuela, and Mexico), despite the fact that *Leishmania major* in commonly found in Old World regions. The aim of this study was to evaluate inter- and intraspecific genetic variability of *L. major* strains and *L. major*-like isolates from Brazil. Identification and characterization of *L. major*-like isolates in Brazil is fundamental in order to establish the state of the eco-epidemiolgy of ACL in the country. Multilocus Microsatellite Typing (MLMT) and gene sequencing of *ITS1*, *hsp*70 and *nagt* of *L. major*-like isolates from Brazil were used to investigate their genetic diversity and to compare them with strains of *L. major* from other Old World countries. Analyses of the 10 microsatellite *loci* previously described for *L. major* and the nucleotide sequences of *ITS1*, *hsp*70 and *nagt* demonstrated inter- and intraspecific genetic variability in the *Leishmania* samples studied. MLMT revealed that *L. major*-like isolates have a different allelic composition. The *L. major*-like isolates exhibited

great genetic diversity, with different homozygous and heterozygous allele combinations. Both phylogentic trees based on the MLMT and gene sequences of *ITS1*, *hsp70* and *nagt* demonstrated complex allelic and genotypic combinations between the strains of *L. major* and the *L. major*-like isolates. There is still a series of complex interactions and the relationship between *L. major* and *L. major*-like parasites is not yet totally understood. *L. major* is a species geographically very distant from Brazil, and with different vectors in the Old World. The possibility of co-adaptation of *L. major* in the New World remains an open question. To distinguish the species of *Leishmania* is one of the key elements of epidemiological studies, in the treatment of disease and in the adoption of adequate control measures in endemic areas for leishmaniasis.

Keywords *Leishmania major*-like; GENETIC VARIABILITY; MICROSATELLITES; GENE SEQUENCING

Financing CNPq and CAPES

P2-083: LIVER GENE SIGNATURE IN THE PROGRESSION OF *Leishmania infantum* INFECTION OF BALB/C MICE

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The parasite-host cell interaction in the infection by *Leishmania infantum*, induces important changes in transcriptome profiles in the parasite but also in the target organs. The detailed analysis of gene expression in infected tissues will contribute to the understanding of the immunological pathways that lead to protection or progression of disease. High-throughput real-time qPCR was used to evaluate the expression of 223 genes related to immunometabolism at different timepoints (1,3,5 and 10 days post infection) of *L. infantum* infection from liver samples of infected BALB/c mice and control mice. Three parameters were studied: parasite load, liver weight and gene expression profile. A multivariate Principal Component Analysis (PCA) was performed to identify gene expression patterns in mice. Gene set enrichment analysis (GSEA) was performed and STRING interaction network was constructed, topological clustering algorithm was applied to determine clusters and functional enrichment analysis was performed. In our model, the multivariate analysis using PCA lead to identify a particular gene expression profile in 10- days infected mice. This differentiation owes to the expression of genes correlated to Principal

Component 3 (PC3). Gene set enrichment analysis applied to PC3 correlated genes revealed that the gene expression signature is characterized by the overrepresentation of *IL12-mediated signaling events* (FDR=0.003) with the upregulation of *Il12rb2*, *Ifng*, *Nos2*, *Il12rb1*, and *Il12b*. The STRING functional enrichment analysis showed some annotations overrepresented: Gene Ontology (GO) processes: *positive regulation of interferon-gamma production* (FDR=3.5E-8) with the upregulation of *Il12rb1, Il12rb2, Il23a, Il21 and Il12b; leukocyte chemotaxis* (FDR= 5.3E-9) with the upregulation of *Ccl7, Cxcr3, Ifng, Ccr7, Cxcr2, Cxcr5.* The transcriptional analysis to approach host–pathogen molecular interactions in experimental visceral leishmaniasis has led to elucidate key genes involved in the progression of the infection in the liver. Our methodology allowed to streamline a large collection of RT-qPCR gene expression data to characterize the most relevant changes at the transcriptome level, overcoming the need of confirmation by RT-qPCR of other methodologies like RNA-seq. In conclusion, the study of gene expression using a global statistical analysis, allowed to decipher the main immunological mechanisms underlying the progression of the infection by *L. infantum* in livers of BALB/c mice. At 10 days post infection, the upregulation of Th1 markers and mediators in IL-12 signaling pathway characterized a well-defined gene signature.

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Keywords TRANSCRIPTIONAL PROFILING; HIGH-THROUGHPUT RT-qPCR; GENE SIGNATURE; PROGRESSION OF INFECTION; *Leishmania infantum*

P2-084: GENOMIC VARIATIONS IN A LABORATORY-INDUCED ANTIMONY-RESISTANT STRAIN OF *Leishmania panamensis*

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Due to the absence of transcriptional regulation of gene expression in *Leishmania* parasites, it is now well accepted that several forms of genomic variations modulate the levels of critical proteins through changes in gene dosage. Many such variations have been reported both in field isolates and laboratory-adapted strains of *L. panamensis* and other *Leishmania* species. These variations not only occur at the level of individual genes but also encompass genetic amplifications in the form of linear or circular episomes and changes in the somy of entire chromosomes as part of a phenomenon called mosaic aneuploidy. These genomic variations have been associated with a better adaptation to environmental conditions in *Leishmania* parasites and with the development of drug resistant phenotypes. In this study, we artificially generated the antimony (Sb)-resistant phenotype in our reference strain of *L. panamensis* (strain PSC-1), through exposure to increasing concentrations of trivalent antimony (Sb^{III}). We then used whole-genome sequencing (WGS) to characterize the genomic changes occurring in the PSC-1 strain after selection at Sb^{III} concentrations of 400, 800, 1600, and 2000 μM, during the process of generating the resistant phenotype. We

observed changes in the somy of several chromosomes, amplifications of several chromosomal regions, and copy number variations in gene arrays after exposure to Sb^{III}. Remarkably, chromosomes 23 and 31, which had already an increased somy in the untreated PSC-1 strain, respectively changed to tetrasomic and hexasomic after exposure to Sb^{III}. We found a notable local amplification in chromosome 23, overlapping the region occupied by the H-locus, a region that has been previously reported to be amplified in resistant strains of *Leishmania*. This amplification appears to occur at concentrations above 800 µM of Sb^{III} and spans a region of ~53 kb, comprising 18 protein-coding genes. Occurrence of amplifications potentially beneficial for the Sb-resistant phenotype appears to be associated with the loss of other forms of amplification, such as a linear minichromosome derived from chromosome 34 and previously reported to be present in the original PSC-1 strain. Although this type of studies are useful for identifying changes in a wide variety of genes under drug selection, potentially identifying novel drug targets, these results do not necessarily reflect the exact genomic changes that could be present in resistant field isolates.

Keywords *Leishmania panamensis*; GENOMIC VARIATIONS; ANTIMONY RESISTANCE

P2-085: GENE EXPRESSION CHANGES IN MACROPHAGES DERIVED FROM MOUSE STRAINS SUSCEPTIBLE AND RESISTANT TO *Leishmania panamensis* INFECTION

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Kinetoplastid parasites of the *Leishmania* genus cause a diverse set of clinical presentations collectively known as leishmaniasis. *Leishmania* parasites exhibit a digenetic life cycle, with an extracellular promastigote form that lives in the sandfly vector and an intracellular amastigote that replicates within macrophages of mammalian hosts. Upon phagocytosis, *Leishmania* parasites develop within phagolysosome-derived structures known as parasitophorous vacuoles, where they delay cytotoxic responses in order to grow and replicate. *Leishmania* parasites can trigger different host immune responses that result in varying levels of disease severity. The C57BL/6 and BALB/c mouse strains are among the host models commonly used for characterizing the immunopathogenesis of *Leishmania* species and the possible antileishmanial effect of novel drug candidates. C57BL/6 mice display resistance to the majority of *Leishmania* infections, due to a predominant Th1 cytokine response and M1 macrophage activation. Conversely, most *Leishmania* infections in BALB/c mice trigger an initial Th2 response conferring a susceptible phenotype that results in sustained parasite growth and tissue damage. Studying species-specific interactions between *Leishmania* parasites and different host systems is a key step to characterize and validate these models for in vivo studies. Here, we use RNA-Seq and differential expression analysis to characterize the transcriptomic profiles of peritoneal-derived C57BL/6 and BALB/c

macrophages in response to *Leishmania panamensis* infection. Additionally, we measured the concentrations of tumor necrosis factor (TNF) and interleukin 10 (IL-10) in supernatants from infected and non-infected peritoneal macrophage cultures using ELISA. For nitric oxide (NO) determination, we used the Griess Reagent System. We observed differences between BALB/c and C57BL/6 macrophages regarding pathways associated with degradation within lysosomes, arginine metabolism and the regulation of cell cycle. We also observed differences in the expression of chemokine, MAPK and cytokine genes associated with regulation of immune responses, including the TNF signaling pathway. Overall, infection with *L. panamensis* induced an inflammatory gene expression pattern in C57BL/6 macrophages that is more consistently associated with a classic macrophage M1 activation, whereas in BALB/c macrophages we observed a gene expression pattern consistent with an intermediate inflammatory response that favors parasite persistence and chronicity of the disease. The intermediate pattern observed in BALB/c macrophages resembles that observed in human infections with *L. panamensis* and supports the use of BALB/c as the preferred model for studying *L. panamensis* infection.

Keywords *Leishmania panamensis*; RNA-seq; DIFFERENTIAL EXPRESSION; SUSCEPTIBILITY; RESISTANCE

P2-086: GENE DELETION AND POST-TRANSCRIPTIONAL REGULATION AS NOVEL DRIVERS OF *Leishmania* EVOLUTIONARY ADAPTATION

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In the absence of transcriptional regulation, *Leishmania* parasites frequently exploit variations in chromosome and gene copy number to regulate expression levels. While gene amplification has been previously linked to positive selection of beneficial phenotypes in culture and in the field (e.g. drug resistance), the role of gene deletion as a possible driver of *Leishmania* adaptation has been less explored. By analysing published *L. donovani* genomes we revealed frequent gene deletion, raising the question of how these parasites cope with such potentially fatal gene copy number variations. Combining experimental evolution and whole-genome sequencing analyses, we identified a spontaneous null mutant for the NIMA-related protein kinase Ld1S_360735700, which penetrated the parasite population during adaptation of hamster-derived amastigotes to in vitro culture. PCR-based detection of this null mutant in the original amastigote isolate indicated that this deletion is not generated de novo but positively selected during culture adaptation, likely providing a fitness advantage. We tested this possibility by re-engineering this deletion in a wild-type population using CRISPR/Cas9-gene editing. Paradoxically, homozygous deletion caused a lethal phenotype as judged by the absence of null mutant parasites. Heterozygous NIMA$^{+/-}$ deletion mutants were viable but strongly affected in cell growth showing an increase in generation time from under 20 to over 80 hours. To better understand how a spontaneous null mutant of an otherwise essential gene can provide fitness gain, we applied genome

and RNA sequencing on a series of NIMA(+) and NIMA(-) clones derived from the original parasite population at passage 20 during culture adaptation. Comparative genomic and transcriptomic analyses identified massive yet highly reproducible changes in RNA abundance in the NIMA(-) clones that were not caused by gene amplification. In the absence of transcriptional gene expression control in *Leishmania*, these results reveal post-transcriptional regulation as a key mechanism to compensate for lethal null mutant phenotypes. Increased RNA abundance was observed for a second NIMA homolog, which may directly substitute for the deleted NIMA gene. Surprisingly, RNA abundance was reduced in the NIMA(-) clones for a series of flagellar transcripts, which correlated with strongly impaired motility. This defect likely represents a fitness trade-off, with the loss of a non-essential function (i.e. flagellar motility) providing the energy necessary for accelerated proliferation, which represents the main fitness phenotype in culture. In conclusion, our results uncover gene deletion and compensatory responses at post-transcriptional level as novel drivers of fitness gain in vitro. These mechanisms significantly increase the parasite fitness landscape and may be relevant for ecological adaptation in the field, as supported by the predominance of a 12kb genomic deletion in *L. infantum* field isolates from Brazil, where these Old-World parasites were introduced during the European conquest ca. 500 years ago.

Keywords *Leishmania donovani*; EXPERIMENTAL EVOLUTION; GENE DELETION; POST-TRANSCRIPTIONAL REGULATION; FITNESS GAIN

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P2-087: DISRUPTION OF *KHARON1* GENE IN *Leishmania infantum* AMASTIGOTES MODULATES mRNAs THAT ENCODES FOR CELL DIVISION/CYTOSKELETON-ASSOCIATED PROTEINS

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The disruption of *kharon1* gene in *Leishmania* spp. leads to live-attenuated amastigotes, impairing cell cycle progression due to cytokinesis defect resulting in parasites unable to maintain infection. This is being explored as a first-generation vaccine strategy against leishmaniasis. Since kharon1 plays an essential role in cell division, Kharon1 (KH1)-deficient *Leishmania* can be applied as a functional tool to better understand the molecular basis of this process, a field poorly explored in these parasites. Here we select *L. infantum kharon1* null mutants (*Likh1*$^{-/-}$) by CRISPR/Cas9 and evaluate, by qRT-PCR, the modulation of transcripts that encode for eight cell division/cytoskeleton-associated proteins: Centrin1, Sfi-1 (spindle body protein), SAS-6 (spindle assembly abnormal protein 6/basal body cartwheel protein 6), KKT (kinetoplastid kinetochore protein), KMP-11 (kinetoplastid membrane protein 11), AIRK (aurora-I related kinase), KHAP1 and KHAP2 (kharon associated proteins 1 and 2). In *Likh1*$^{-/-}$, Centrin1, KHAP1 and KHAP2 were upregulated, while SAS-6 was downregulated, which may indicate compensatory and/or dependent actions. This feature was absent in promastigote forms, being detected only in intracellular amastigotes; the evolutive form in which kharon1 is essential. We can hypothesize that during cell division kharon1 play a role dependent of these modulated factors which are probably orchestrating cell division dynamics in *L. infantum*. Whether they act as a protein complex or regulating (direct or

indirectly) cell division is yet to be described. Curiously, when disrupted, KH1, Centrin1, KMP-11, KHAP1 and KHAP2 (independently), leads to a similar phenotype of live-attenuated multinucleated parasites. Kharon1 localizes in the flagellar pocket area and extends surrounding the parasite cell body either in *L. infantum* and *L. braziliensis*. Further analyses are being performed to evaluate the role of kharon1 in *Leishmania* cell division. The basic findings here can be applied to better understand the mechanisms of chromosomal segregation which is very particular in *Leishmania* parasites and can be used as a model of mosaic aneuploidy. Additionally, these proteins can be studied as drug targets, supporting the development of alternative drugs for tackling leishmaniasis.

Keywords *Leishmania*; KHARON1; CELL DIVISION

Financial Support Fapemig, CAPES, CNPq, Fiocruz

P2-089: DEPLETION OF A SINGLE IP6K ALLELE ALTERS CELL MORPHOLOGY AND LEAD PART OF THE *Trypanosoma cruzi* POPULATION TO DORMANCY

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Inositol pyrophosphates (PP-IPs) – mainly IP_7, and IP_8 – are involved in a wide range of processes in eukaryotes. However, the mechanism of action of PP-IPs is not fully understood yet. IP_7 and IP_8 are synthesized by pathways involving the participation of IP6K and PP-IP5K kinases, respectively. Trypanosomatids have an ortholog gene for IP6K, but apparently do not have orthologs for PP-IP5K, *i.e.*, they probably do not synthesize IP_8, which makes them excellent models for the study of IP_7. Using the CRISPR/Cas9 system and two rounds of sgRNA transfection, we were able to deplete the single and double alleles of IP6K in *Trypanosoma cruzi* (the causative agent of Chagas Disease), generating IP6K$^{-/+}$ and IP6K$^{-/-}$ lineages, respectively. Removal of IP6K causes several morphological effects in both lineages, such as rounding and wrinkling of the cell body, increased number of glycosomes, and mitochondrial enlargement. Notably, IP6K$^{-/-}$ lineage was unable to proliferate, and most *T. cruzi* cells died a few days after transfection, suggesting IP6K is essential to this organism. Curiously, IP6K$^{-/+}$ lineage showed a slight cell cycle arrest at G0/G1 phase. However, the arrested population showed no DNA damage. Then, we estimated the cell cycle phases length and the doubling-time in *T. cruzi*, and used them to develop a pioneering assay to measure quiescent (dormant) cells based on negative EdU (5-Ethynyl-2′-deoxyuridine) labeling. Our preliminary data suggest that the partial depletion of IP6K leads part of *T. cruzi* population to dormancy. Our next steps include performing measurement of metabolites (including IP_7 levels), metacyclogenesis induction and evaluation of infection capacity in the *T. cruzi* IP6K$^{-/+}$ lineage. Our data is clarifying the

general effects of loss of IP6K in the human pathogen *T. cruzi*, which will contribute significantly to a better understanding of the pyrophosphorylation performed by IP_7, a non-enzymatic post-translational modification still little studied.

Keywords CRISPR/CAS9; INOSITOL PYROPHOSPHATES; *Trypanosma cruzi*; QUIESCENCE

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P2-092: EFFECTS OF MEIOSIS-RELATED GENES DELETION IN *Leishmania* HYBRIDIZATION

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The protozoan parasites from the *Leishmania* genus are the causative agents of leishmaniasis, a vector-borne disease that ranges from self-healing cutaneous lesions to life threatening visceral infections. Clinical outcomes of the disease are known to be related to species diversity and the emergence of new strains. Previously, a cryptic sexual cycle has been described involving the extracellular promastigote stages developing in the sand fly vector. The generation of intra- and interspecific hybrids was demonstrated in laboratory crosses during sand fly infections and, more recently, in culture after DNA damage induced by gamma-irradiation. Although no gametes or meiotic forms have been identified, allele inheritance patterns strongly suggest a meiotic process. Using single cell RNA sequencing, we previously identified a subgroup of DNA stressed cells that upregulated a number of meiosis-related genes, including the ancestral gamete fusogen HAP2. Here, we used *L. tropica* parental strains MA37 and L747 that have a high mating efficiency to generate CRISPR-Cas9 competent cell lines and to delete the meiosis-related genes HAP2-1, HAP2-2, SPO11, MND1, DMC1, HOP1 and HOP2, to investigate their respective roles in genetic exchange. We were able to generate null mutants for each of these genes in both L747 and MA37 by substituting the whole CDS for the Puromycin N-acetyltransferase (PAC) gene flanked by 5' and 3' UTRs of a *Leishmania* housekeeping gene. We used the null mutants from one of the strains in combination with a control line containing the Blasticidin S deaminase (BSD) gene integrated into the SSU rRNA locus (e.g.: MA37 ΔHAP2-PAC and L747 SSU-BSD), and selected for hybrids resistant to both PAC and BSD. When null mutants of *L. tropica* MA37 were used for *in vitro* crosses, only ΔHAP2con-2 showed a significant decrease in the minimum frequency of

hybridization-competent cells (5.8-fold lower, p = 0.0021). By contrast, L747 null mutants showed a reduction for ΔHAP2-2 (1.5-fold lower, p < 0.0001), ΔDMC1 (1.9-fold lower, p < 0.0001), ΔHOP2 (5.5-fold lower, p=0.0003) and ΔHOP1, for which no hybrids were recovered in any of the 3 replicate experiments. Preliminary results using *Lutzomyia longipalpis* sand flies indicated that the *L. tropica* L747 ΔHOP1 mutant was impaired for hybridization *in vivo.* These findings implicate the involvement of several protein components of the meiotic machinery, including plasmogamy, synaptonemal complex formation, and recombination, in *Leishmania* hybridization. Further experiments, including the generation and testing of re-expressor lines, are being performed to further investigate the role of meiosis-related genes in genetic exchange.

Keywords *Leishmania tropica;* DNA; HAP2-1; HAP2-2; SPO11; MND1; DMC1; HOP1; HOP2

P2-093: COMPARATIVE CHARACTERIZATION OF GENOMIC VARIABILITY OF *Leishmania* spp. ISOLATES OBTAINED FROM DOGS WITH VISCERAL LEISHMANIASIS IN DIFFERENT ENDEMIC AREAS IN BRAZIL

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Leishmaniasis is a complex of diseases caused by protozoan parasites of *Leishmania* genus, endemic in Brazil and in countries of the Old and New worlds. The clinical manifestations of the disease depend on the infectivity of the species involved and the host immune response. *Leishmania* (*Leishmania*) *infantum* is associated with the visceral form of the disease in humans and dogs, but some studies evidence the potential of visceralization of *Leishmania* (*L.*) *amazonensis*. It is known that these trypanosomatids have great potential of adaptability in the face of the most adverse environmental conditions. The biological cycle of these parasites can undergo changes due to the environment in which they are exposed and, mainly, by anthroponotic changes in the ecosystem. The survival and perpetuation of *Leishmania* are related to modifications at genetic level, which can modify its gene expression. Hence, there is great relevance in employing field samples from

hosts, such as dogs and humans, from endemic areas for visceral leishmaniasis (VL), where parasites certainly undergo environmental pressures and can alter their genomic content and eventually their gene expression due to reservoirs, vectors and hosts availability. This work aims at performing a comparative analysis of *Leishmania infantum* isolates, predominantly from naturally infected dogs from different states of Brazil – Bahia, Minas Gerais (Januária and Governador Valadares cities), Rio Grande do Norte (RN) and Santa Catarina (SC). By applying bioinformatic tools, 85 genomes were analyzed for variation of chromosome copy number and SNVs, phylogeny, population structure and gene enrichment. The SNPs and phylogeny analyses indicated a pattern of clusterization by endemic region. Intra and interpopulation variations were observed among the isolates. Januária isolates formed two well defined clades, one with rural and the other with urban isolates, suggesting that distinct transmission cycles are occurring in the area. Also, isolates from Januária and RN have little intra and interpopulation variability; Governador Valadares and SC isolates have a higher frequency of heterozygous genotypes when compared to Januaria and RN isolates. In addition, *in silico* analyses also revealed aneuploidies more evident on chromosomes 8, 23 and 31, which contain enriched genes related to the metabolic activities, survival and virulence of the parasites. This study suggests that genomic surveillance of *Leishmania* is of great relevance for a better understanding of disease dynamics in endemic regions.

Keywords VISCERAL LEISHMANIASIS; DOGS; *Leishmania* spp.; GENOMIC VARIABILITY; ADAPTABILITY

P2-094: UNDERSTANDING THE EFFECTS OF TELOMERASE RNA (TER) KNOCKOUT IN *Leishmania tropica*

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Leishmaniases, caused by *Leishmania* spp., are global public health threats, affecting millions worldwide. The disease presents different clinical outcomes and a high incidence rate without effective prevention and treatment protocols. Thus, finding new therapeutic targets is crucial to eradicating the disease. Telomeres, the nucleoprotein structures found at the end of most eukaryotic chromosomes, have been considered potential targets against the disease. In most eukaryotes, telomeres are key to maintaining genome stability by avoiding chromosome ends from being recognized as DNA double-stranded breaks. They are maintained by the telomerase ribonucleoprotein complex minimally composed of Telomerase Reverse Transcriptase (TERT) and Telomerase RNA (TER). TERT uses the TER component template sequence to synthesize telomeric DNA at the 3' end of the G-rich strand. TER is also involved in telomerase assembly and processivity. Since TER can greatly vary in size and sequence among the organisms, it can be considered a potential target against *Leishmania* spp. Therefore, our goal is to study the effects of TER knockout in *Leishmania major* life span and development. LeishTER double-knockouts (LmTER$^{-/-}$) were achieved using the CRISPR-Cas9 system. Double knockout was confirmed by PCR, RT-PCR, Southern blot, and automated sequencing using wild-type (wt) parasites as the control. Analysis of the LmTER$^{-/-}$ growth profile showed that after five passages in culture (P5), the parasites showed lower cell concentration (cells/ml) and a longer stationary phase. However, in late passages (P35 and P50), LmTER$^{-/-}$ showed a concentration of cells/ml and a growth profile in the exponential phase, very similar to the control, with few changes in the stationary phase. In addition, the proliferation analysis using CFSE (carboxyfluorescein succinimidyl ester) showed mild

alterations in cell division. Also, with continuous *in vitro* passages, LmTER$^{-/-}$ showed a partial arrest in the G0/G1 cell cycle phase, progressive telomere shortening, and increased DNA damage signaling with the detection of phosphorylated histone H2A (γH2A). However, the parasites were still able to differentiate into metacyclics, the infective form of the parasite. We are investigating the infectivity capacity of LmTER$^{-/-}$ and intend to do a long-term observation about the impact of telomere shortening on the parasite's ability to respond to DNA damage, environmental changes, and development and survival.

Keywords *L.* major; LEISHTER; GENE EDITING; CELL PROLIFERATION; TELOMERE SHORTENING

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P2-095: THE COMPLETE SEQUENCE FOR *Leishmania aethiopica* GENOME AND MAXICIRCLE AS DETERMINED BY A COMBINATION OF SEQUENCING METHODOLOGIES

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Leishmania aethiopica is the main causative agent of cutaneous leishmaniasis (CL) in Ethiopia. Facial lesions in patients infected by this parasite are more severe and take longer to cure than those produced by other *Leishmania* species. Moreover, diffuse cutaneous leishmaniasis and mucocutaneous manifestations are quite common too. The parasitological features that underlay these severe clinical presentations remain unknown. The availability of a complete and well-annotated genome will contribute to get a better knowledge of this species and will contribute to develop strategies for diagnosis and disease control. Promastigotes of *L. aethiopica* MHOM/ET/1972/L100 strain (aka L147), were obtained from WHO Collaborating Centre for Leishmaniasis, Centro Nacional de Microbiología (Instituto de Salud Carlos III (ISCIII), Madrid, Spain). DNA was obtained by classical phenol and chloroform/isoamyl alcohol extraction. Single-molecule real-time (SMRT) sequencing technology developed by Pacific Biosciences (PacBio) was performed at the Norwegian Sequencing Centre hosted by the University of Oslo. Additionally, library construction and paired-end sequencing (2 x 150 bp) using Illumina NovaSeq technology were performed at the Genomics Unit of ISCIII. *De novo* genome assembly was carried out by the Genomics and NGS Core Facility (GENGS), at the

Centro de Biología Molecular Severo Ochoa (CBMSO, CSIC-UAM) using a novel workflow to combine data generated by both sequencing methodologies. In total, 106,905 PacBio HiFi reads (11,220 pb mean length) were assembled using HGAP (59 contigs) and Canu software (187 contigs). Based on the genome size (30.98 Mb) for the *L. aethiopica* draft-assembly (currently available at TriTrypDB), an average sequencing depth of 38x was attained. Twenty-seven of these contigs were found to correspond to complete chromosomes. Sequences for the other nine chromosomes were initially found in two or more contigs, but they could be joined with SSPACE tool using Illumina reads and Scaffold_builder software. Additionally, a total of 47,387,024 Illumina reads (average sequencing depth of 461x) were assembled using SPADES software into 6,285 contigs that were aligned against the PacBio assembled chromosomes using MAFFT and LAST aligners. This process was useful to extend chromosome ends. Furthermore, Illumina reads were also employed to correct 1,763 bases erroneously inserted and 106 bases erroneously deleted in PacBio sequencing reads; this correction was done using the ARAMIS pipeline. Finally, the 36 chromosomes of the assembled genome (32,868,683 bp) were annotated using the Companion software. In parallel, the maxicircle sequence of the kinetoplast DNA was assembled from PacBio reads and then corrected based on the Illumina reads. The combination of sequencing platforms providing short and long reads has enabled to assemble, without any sequence gap, the complete 36 *L. aethiopica* chromosomes. This *de novo* genome assembly for *L. aethiopica* represents an improvement regarding the current genome version available in public databases for this species. The availability of a reliable reference genome constitutes the cornerstone to undertake studies aimed to analyze differential gene expression by transcriptomic and proteomic approaches that will serve to decipher phenotypic peculiarities such as tissue tropism, clinical disease, and drug susceptibility.

Keywords *Leishmania aethiopica*; GENOME ASSEMBLY; DNASEQ; MAXICIRCLE; NEXT-GENERATION SEQUENCING (NGS)

P2-096: INSIGHTS INTO THE GENETIC DIVERSITY OF *Leishmania panamensis* IN PANAMA INFERRED BY MULTILOCUS SEQUENCE TYPING

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Leishmaniasis is a group of diseases caused by protozoa parasites of *Leishmania* genus and transmitted by sand fly vectors with an eminent neglected status. In Panama, *Leishmania panamensis* is responsible for nearly 80% of the tegumentary form of the disease; clinical outcome that presents different clinical expression. It is though that one of the elements responsible for the final clinical outcome of Leishmaniasis is the parasite genetic make-up. In this sense, there are little studies describing the genetic diversity of *L. panamensis* in Panama and most of them assessed the diversity of this species using a single molecular marker or a small sample number. In this study we assessed the genetic make-up of 69 *L. panamensis* isolates from recognized endemic areas of the country using a MLST scheme based on 4 housekeeping genes (HSP70, Aconitase, ALAT and GPI). We used the DNAsp software to construct haplotypes from each molecular marker and assess diversity index by region and locus used herein. The optimal number of markers, evaluation of topological incongruency and estimation of *L. panamensis* diploid sequence type (DSTs) were performed with the MLSTest software. We confirmed the status of DSTs obtained by MLSTest as genotypes of *L. panamensis* by Phylogenetic analysis using MrBayes v.3.2. The clonal complex analysis of *L. panamensis* isolates was carried out by the eBurst algorithm within the Phyloviz 2.0 package. To estimate the global ancestry of the local *L. panamensis* isolates we used STRUCTURE v.2.0. At haplotype level, we found between two to seven *L. panamensis* haplotypes

depending on the loci employed and differences in diversity indexes and haplotyped diversity between East and Western regions of the country when Aconitase and GPI were used as markers. Our results point out the circulation of 13 *L. panamensis* genotypes some of them restricted to specific regions of the country. We also found that DST1 is the most prevalent and the founder genotype of the *L. panamensis* clonal complex identified in this study. All genotypes showed to came from an admixture ancestral and presented different individual admixture proportions that might be result of inheriting unequal's allelic proportions from the postulated ancestral population. The presence of different *L. panamensis* genotypes circulating in the country might be associated with the different clinical expressions of the tegumentary form caused by this species, and therefore could have great impact in the patient's management and disease control.

Keywords *Leishmania panamensis*; MULTILOCUS TYPING; GENETIC DIVERSITY; DIPLOID SEQUENCE TYPE; PANAMA

P2-097: EFFECTS OF A FREQUENT GENOMIC DELETION AMONG BRAZILIAN *Leishmania infantum* STRAINS

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Leishmania infantum (syn. chagasi) is a non-native protozoan parasite, introduced in Brazil during European colonization. Once in the New World (NW), these imported strains successfully adapted to new vectors and established new transmission cycles. Due to the recent arrival in the Americas, genomic changes might be present, due to allele surfing and/or selection by the new environment. Indeed, our group reported a genomic trait widespread and frequent among Brazilian strains. A 12Kb deletion at the stable, supernumerary chr31. One of the ORF within the deletion site encodes the Ecto-3'-nucleotidase, an important enzyme for the uptake of purines by the parasite. Moreover, this transmembrane enzyme constitutes a virulence factor in *L. infantum*. It was recently shown by us a significant reduction or absence of Ecto-3'-nucleotidase activity in promastigotes DEL, when compared to NonDEL and heterozygous (HTZ). This enzyme has an important role during *Leishmania* interaction with neutrophil networks

(NETs); indeed, we previously confirmed promastigote samples carrying the deletion (DEL) survive less than NonDEL strains after the interaction with NETs and infect less macrophages after 24 hours. Based on this, we aimed to investigate further how this difference in Ecto-3'-nucleotidase enzyme activity affects biological characteristics of DEL and NonDEL Brazilian strains. We quantified the percentage of metacyclic promastigotes by PNA, of strains with 10-15 passages (10-15P) and with >25 passages (>25P). DEL strains with either >25 passages or <10-15P, presented a higher percentage of metacyclics in relation to the NonDEL strains; as expected, samples with fewer passages exhibit higher metacyclic compared to its >25P counterpart. The Ecto-3'-nucleotidase activity was measured in PNA+, PNA- fractions confirming the higher activity of PNA- (metacyclics). Amastigotes recovered from spleen and liver of infected hamsters will further be analyzed for Ecto-3'-nucleotidase activity. Ecto-nucleotidases generate purines, such as adenosine (ADO). Extracellular ADO affects the purinergic signaling pathway of the host, creating an anti-inflammatory environment in macrophages and dendritic cells. These results give us a glimpse of potential biological differences between the studied strains. We thus hypothesize that DEL strains and its associate reduced Ecto-3'-nucleotidase activity generates less ADO at the site of infection, leading to a less virulent and pathogenic profile - a feature that favors parasite spread, i.e., less severe disease or asymptomatic hosts, which would remain longer periods as source of infection for vectors. To contribute to test the raised hypothesis, we aim to further asses the outcome of infection by the DEL, NonDel strains in animal models. We are currently determining the parasite load of spleen and liver of 3 weeks infected BALB/c mice, histopathology analysis and assessing the levels of ADO and adenosine deaminase (ADA) in these animals. Furthermore, the recruitment of neutrophils and vascular alterations in the skin of BALB/c 24 hs PI were evaluate by intravital video-microscopy and the data is under analysis. In this way, we aim to biologically characterize these Brazilian genotypes of *L. infantum*, and contribute to elucidate of how these genomic alterations are impacting the eco-epidemiological scenario of the parasite in the NW.

Keywords *Leishmania infantum*; BRAZIL; VISCERAL LEISHMANIASIS

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P2-098: GENOMIC VARIABILITY OF COLOMBIAN STRAINS OF *Leishmania braziliensis* AND *L. guyanensis*

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Cutaneous leishmaniasis (CL) is an endemic infectious disease in Colombia producing about 10,000 cases/year mainly associated with *Leishmania* (V) *panamensis*, *L.* (V) *guyanensis* and *L. (V) braziliensis*. In this study we report the preliminary results of the whole genome sequencing of clinical isolates of *L. (V) braziliensis* (n=22) and *L.*(V) *guyanensis* (n=3) and a comparative analysis regarding SNPs and structural variations. Genomic DNA was prepared from promastigotes kept in Schneider media with few culture passages (<20) after the patient's isolation. Sequencing was performed throughout the Illumina platform, once the quality of reads was verified, then reads were aligned against the reference strain genome M2904 of *L. (V) braziliensis*. Mapped reads were used to assess diversity by analyzing the variation in ploidy, variation in copy number and SNPs, using the NGSEP software. Subsequently, the presence of subpopulations or subgroups in *L.(V) braziliensis* strains was evaluated based on SNPs variability. The ploidy analysis showed that the 25 sequenced Colombian clinical isolates are predominantly diploid as previously reported for L.*(V) panamensis*, except for the chromosome 31, which has double the number of copies as in other *Leishmania* spp. However, chromosome 33 showed a slight aneuploid variation in some strains. Furthermore, the presence of a relatively large fragment with a uniform increase in read depth on chromosome 34,

spanning approximately 60 kb and 13 genes, was identified in 19 of the *L. (V) braziliensis* isolates and in 1 of L. *(V) guyanensis isolate*. Additionally, one *L.(V) braziliensis* isolates showed an increase in read depth on the end of the chromosome 34 that covers about 140 kb and 38 genes. Previously, the presence of amplification of the fragments above described on chromosome 34 had been reported in *L.(V) panamensis* and *L.(V) braziliensis* M2903. These regions are likely to be amplified, either duplicated within the chromosomes or as extrachromosomal elements. The first amplified fragment encompasses 13 genes of unknown function, apart from putatively encoding a protein of the SMC (structural maintenance of chromosome) family and Phosphatidylinositol transfer protein-like involved in survival. The second one contains the LD1 region. This LD1 region includes the BT1 gene encoding a biopterin transporter. Accordingly to the SNPs analysis, the isolates groups in two big groups congruently with their species, *L.(V) braziliensis* in the first group clustering with reference strain M2904 and *L.(V) guyanensis* in the second one, grouping with Colombian and Panamanian isolates of *L.(V) panamensis* obtained from the Bioproject database. Interestingly, the SNPs analysis did not group the clinical isolates regarding their geographical origin. The results acquired here suggest that the amplification fragments do not appear to be a constitutive phenomenon in all leishmania Viannia Colombian strains.

Keywords *Leishmania (V) braziliensis; L. (V) guyanensis*; STRUCTURAL VARIATION; GENOMIC DIVERSITY

P3-079: BIOCHEMICAL AND FUNCTIONAL CHARACTERIZATION OF A NEW PUTATIVE SERINE/THREONINE KINASE (AKT-LIKE) FROM *Trypanosoma cruzi*

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There is currently a need to find new drug therapies for Chagas disease, a systemic infection with no effective cure in its chronic stage due to the low efficiency and the harmful side effects of the few drugs available against it. Protein kinases are promising drug targets due to their essential role in cell signaling pathways. In this scenario, we propose the identification and functional validation of a protein associated with PI3K/AKT/mTOR signaling pathway as a potential drug target, a possible second serine/threonine kinase B-like protein of the *T. cruzi* parasite. By bioinformatics analysis, we found that the genome of *Trypanosoma cruzi*, in addition to the first reported AKT-like (UniProtKB ID Q4D6D3), harbors another sequence similar to AKT-like but with divergence at the N-terminus (FYVE/PHD), a domain that is not present in any of the three human AKT isozymes and whose sequence has a maximum identity of 41% with the human FYVE/PHD domains; a fact that allows the rational design of compounds that take advantage of these differences with the parasite. Using molecular biology tools: plasmids pETZ2-AKT-2 and pET28a-AKT-2 were obtained, which allowed the expression of the recombinant AKT2-like with a 6His tag in the heterologous system of *Escherichia coli*. Purification was performed by immobilized metal affinity chromatography, ionic exchange, and size exclusion chromatography. The kinase activity was evaluated using a solid phase enzyme-linked immunosorbent assay (ab139436). Two rabbits were immunized with the recombinant AKT2-like, and the antibodies obtained allowed detection of the endogenous protein in *T.*

cruzi epimastigotes by Western-blot assays. In addition, 3D modeling, docking, virtual screening, and molecular dynamics studies were performed. The AKT-2 sequence was subjected to different modeling methodologies (ModWeb, SwissModel, I-TASSER, and the trRosetta server). To execute the molecular docking with AKT-2 (binding sites: FYVE and Pleckstrin domains) dataset of 600K compounds with drug-like properties from the ZINC database was selected. The protein and ligands were parameterized with the AutoDock Tool, and a consensus docking, and sorting protocol called dockECR was used. To check the stability of the selected AKT-2 model, the structure was subjected to a molecular dynamics (MD) simulation of 80 nanoseconds (ns). In conclusion, AKT2-like protein was purified by coupling different chromatographic techniques, its kinase activity was determined, and the protein was immunodetected in *T. cruzi* epimastigotes extracts. The best calculated AKT-2 model was obtained with trRosetta. We observed stability of the PH, AGC and protein kinase domain but the N-terminal FYVE domain fluctuates around the long alpha helix that connects the FYVE and PH domains. In spite of this, the terminal part conserves its secondary structure, and the protein reports a stable RMSD at the end of the simulation. With the molecular docking results, 16 molecules from the FYVE domain and 7 molecules from the PH domain were selected. The *in silico* and experimental results allowed us to validate the existence of a second serine/threonine kinase B-*like* in *T. cruzi* and the proposed molecules are suitable candidates for further experimental validations.

Keywords *Trypanosoma cruzi*; KINASE; AKT/PKB, CHAGAS

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P3-080: BIOPHYSICAL AND BIOCHEMICAL CHARACTERISATION OF THE INTERACTION BETWEEN *Leishmania braziliensis* PRMT1 AND PRMT3

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Arginine methylation is a key post-translational modification that can alter the structure, dynamics and interaction profiles of proteins. Protein arginine methyltransferases (PRMTs) catalyse the transfer of a methyl group from a *S*-adenosylmethionine molecule onto the arginine side chain guanidino group. Mammalian PRMTs are classified into subtypes – PRMT1, 2, 3, 4, 6 and 8 catalyse asymmetric dimethylation (ADMA); PRMT5 and 9 catalyse symmetric dimethylation (SDMA); and PRMT7 catalyses monomethylation (MMA). Kinetoplastids possess five homologues: PRMT1, 3, 5, 6 and 7. *T. brucei* PRMT3 has been shown to be a pro-enzyme (prozyme) which lacks key conserved motifs including in the catalytic double E loop. *T. brucei* PRMT1 is only active in complex with the PRMT3 prozyme. In *Leishmania*, however, PRMT3 retains the conserved double E loop, which raises questions about its role in this organism. Here we use recombinant protein samples to investigate *L. braziliensis* (*Lbr*) PRMT1 and 3 *in vitro*. Activity assays show that methylation of a substrate peptide only occurs when PRMT1 and 3 are both present. Analytical size exclusion chromatography (SEC) show that *Lbr*PRMT1 and 3 form a heterotetrameric complex in solution. Mutation of double E loop residues revealed that *Lbr*PRMT1 is the active component of the complex. Previous work suggested *Lbr*PRMT3 could interact with and modulate the activity of other *Lbr*PRMTs, however methyltransferase assays showed that *Lbr*PRMT3 had no effect on the activities of *Lbr*PRMT5 and 7 *in vitro*. Moreover, *Lbr*PRMT3 could not methylate a peptide substrate previously monomethylated with PRMT7. Our data suggests that *Lbr*PRMT1 and 3 form a similar complex to *T. brucei* PRMT1-3. However, the retention of the conserved double E loop in

Leishmania PRMT3 enzymes suggests an as yet undiscovered functional difference between the two trypanosomatids.

Keywords ARGININE; METHYLATION, PRMT1; PRMT3; OLIGOMERISATION

P3-081: FATTY ACID PROFILES OF *Leishmania major* DERIVED FROM HUMAN AND RODENT HOSTS IN ENDEMIC CUTANEOUS LEISHMANIASIS AREAS OF TUNISIA AND ALGERIA

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Leishmaniasis is a protozoal vector-borne disease that affects both humans and animals. In the Mediterranean Basin, the primary reservoir hosts of *Leishmania* spp. are mainly rodents and canids. Lipidomic approaches have allowed scientists to establish *Leishmania* spp. lipid profiles for the identification of cell stage specific biomarkers, drug mechanisms of action, and host immune response. Given the crucial role of lipids and their variability in both promastigote and amastigote forms of *L. major*, lipidomic profiles of parasites deriving from different hosts are missing, although host and parasite nature have a massive impact on lipid composition and homeostasis. Using an in-silico approach of global network interaction between genes involved in fatty acid (FA) synthesis followed by the GC-MS approach, we were able to characterize the fatty acid profiles of *L. major* derived from human and rodent hosts. Our results revealed that the lipid

profile of *L. major* showed similarities and differences with those already reported for other *Leishmania* species, like the predominance of Phospholipids class. FA composition of rodent parasites was characterized by a lower abundance of the precursor C18:2(n-6). One of the rodent clones, which also expressed the lowest lipid abundance in PL and TAG, was the least sensitive clone to the miltefosine drug and has the lowest infection efficiency. Our findings suggest that the lipid composition variation may explain the response of the parasite toward treatment and their ability to infect their host.

Keywords *Leishmania*; PROMASTIGOTE; HUMAN; RODENT; LIPIDS; FATTY ACIDS

P3-083: ROLE OF TRYPANOTHIONE METABOLISM IN ANTIMONY UNRESPONSIVE OF *Leishmania tropica* CLINICAL ISOLATES

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Clinical resistance to pentavalent antimonial compounds has long been recognized as a major problem in the treatment of human leishmaniasis. Trypanothione metabolism, the main form of thiol, has shown to play a central role in antimony resistance of laboratory-generated resistant *Leishmania* spp. and field-isolated resistant *L. donovani*; but the mechanism of antimony resistance in the clinical isolates of *L. tropica* causing anthroponotic cutaneous leishmaniasis (ACL) is less studied. Patients were selected among confirmed positive ACL cases who referred to Pasteur Institute of Iran, Tehran, from endemic regions of north-east and south of Iran. *L. tropica* clinical isolates were collected from patients who were either treatment-responsive (MAS=S1 to S5) or unresponsive (MAR=R1 to R4) to Glucantime® (meglumine antimoniate=MA). Isolates were tested for sensitivity to trivalent antimony (SbIII) in promastigotes and to pentavalent antimony (SbV) in intracellular amastigotes stages. Intracellular thiol levels were assayed and trypanothione-dependent components, including trypanothione reductase (TR) and tryparedoxin peroxidase I (TryP) were analysed at protein level and enzymatic activity in isolates. The MAR isolates had an approximate two fold increase in the levels of intracellular thiols ($P< 0.05$) accompanied by an average 5-10 fold increase in *in vitro* resistance to antimony. TryP was amplified at the protein level in all MAR strains as compared to the MAS strains (range: 2.8-5.6 fold). All MAR isolates metabolized H_2O_2 at higher rates than MAS isolates (8.55±0.75 nmol/min/mg *vs.* 3.14±0.36 nmol/min/mg) ($P< 0.05$). In addition, levels of TryR protein were also markedly elevated in 3 out of 4 MAR isolates (range: 2.2-4.1 fold). This was accompanied by overexpressed TryR activity (mean level of 46.83±2.43 for extracts of MAR *vs.* 20.98±3.02 for MAS strains) ($P< 0.05$). Elevated levels of TryP, active enzyme in peroxide detoxification, were observed in MAR parasites resulting in an increased metabolism of

H_2O_2. TryR activity was overexpressed on average in extracts of MAR strains, but not in all isolates. Enhanced anti-oxidant defenses through thiol metabolism may play a significant role in clinical resistance of ACL patients to Glucantime.

Keywords ANTHROPONOTIC CUTANEOUS LEISHMANIASIS; THIOL; MEGLUMINE ANTIMONIATE; ANTIMONIAL RESISTANCE; TRYPAREDOXIN PEROXIDASE

P3-084: VARIATION IN THE EXTRACELLULAR CONCENTRATION OF L-ARGININE AFFECTS THE PHYSIOLOGY OF *Leishmania (Viannia) braziliensis* AND ITS SUSCEPTIBILITY TO ANTILEISHMANIAL DRUGS.

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Amino acid metabolism in trypanosomatids is a valuable source of new therapeutic targets. L-arginine (L-arg) is an essential amino acid for *Leishmania* parasites, and it participates in the synthesis of polyamines, a group of essential nutrients used for nucleic acids and proteins biosynthesis necessary for proliferation. In the present study, we showed that the absence of L-arg in culture medium negatively influences the growth and infectivity of *Leishmania (Viannia) braziliensis*, causing a decrease in the percentage of the infected cells and parasite load. The absence of L-arg resulted in the parasite's inability to regulate its reactive oxygen species (ROS) production, which persisted for up to 24 hrs. Moreover, the differentiation of promastigote to amastigote in axenic culture was more significant at low concentrations of L-arg. No association was established between the availability of L-arg and the effectiveness of antileishmanial drugs. All these results confirm the importance of L-arg in *L. braziliensis* life cycle vital processes, such as its replication and infectivity, as documented in other Leishmania species. Based on these results, we proposed that the L-arg uptake/metabolism route is a possible factor in exploring new antileishmanial drugs.

Keywords LEISHMANIASIS; METABOLISM; AMINO ACID; L- ARGININE; CULTURE GROWTH; INFECTIVITY; HPLC-MS

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P3-085: NITRIC OXIDE RESISTANCE IN *Leishmania (Viannia) braziliensis* INVOLVES REGULATION OF GLUCOSE CONSUMPTION, GLUTATHIONE METABOLISM AND ABUNDANCE OF PENTOSE PHOSPHATE PATHWAY ENZYMES

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In American Tegumentary Leishmaniasis production of cytokines, reactive oxygen species and nitric oxide (NO) by host macrophages normally lead to parasite death. However, some *Leishmania braziliensis* strains exhibit natural NO resistance. NO-resistant strains cause more lesions and are frequently more resistant to antimonial treatment than NO-susceptible ones, suggesting that NO-resistant parasites are endowed with specific mechanisms of survival and persistence. To tests this, we analyzed the effect of pro- and antioxidant molecules on the infectivity in vitro of *L. braziliensis* strains exhibiting polar phenotypes of resistance or susceptibility to NO. In

addition, we conducted a comprehensive quantitative mass spectrometry based proteomics analysis of those parasites. We were able to compare those parasites before and after stimulus with an NO donor, identifying ~6300 proteins and estimating absolute concentrations of ~6000 of these proteins. NO-resistant parasites were more infective to peritoneal macrophages, even in the presence of high levels of reactive species. Principal component analysis of protein concentration values clearly differentiated NO-resistant from NO-susceptible parasites, suggesting that there are natural intrinsic differences at molecular level among those strains. Upon NO exposure, NO-resistant parasites rapidly modulated their proteome, increasing their total protein content and glutathione (GSH) metabolism. Furthermore, NO-resistant parasites showed increased glucose analogue uptake, and increased abundance of phosphotransferase and G6PDH after nitrosative challenge, which can contribute to NADPH pool maintenance and fuel the reducing conditions for the recovery of GSH upon NO exposure. Thus, increased glucose consumption and GSH-mediated redox capability may explain the natural resistance *of L. braziliensis* against NO.

Keywords *Leishmania braziliensis*; NITRIC OXIDE RESISTANCE; AMERICAN TEGUMENTARY LEISHMANIASIS; QUANTITATIVE PROTEOMICS

P3-086: DELVING IN ONE CARBON METABOLISM IN THE PARASITE *Leishmania* THROUGH A CHEMI-GENOMIC SCREEN

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Studies of *Leishmania* resistant to the model antifolate drug methotrexate (MTX) has illustrated major differences in one carbon metabolism between the parasite and its host. Some of those differences could be exploited. Genomic screens are now allowing holistic views of metabolic pathways and we apply here our recently optimised Mut-seq screen with MTX selection to further our understanding of one carbon metabolism in *Leishmania*. Mut-seq consists in chemical mutagenesis (with ethyl methanesulfonate) of a *Leishmania* population and its selection on plates containing MTX. Resistant clones are characterized by next-generation sequencing and recurrent mutations (single nucleotide polymorphisms (SNPs), copy number variations), highlighted by a bio-informatics pipeline, in independent mutants are pointing at likely candidates. Candidate mutations are studied by molecular means. Twenty clones of *L. major* with a 2-400-fold decrease in MTX susceptibility in comparison to wild type (WT) cells were sequenced. Recurrent mutations (SNPs, gene deletion) were observed in dihydrofolate reductase thymidylate synthase (DHFR-TS), pteridine reductase 1 (PTR1), several transporter of the folate biopterin transporter (FBT) family, as well as several genes involved in folate metabolism (FPGS, SHMT), as well as genes never associated with folate metabolism. In three mutants we also observed gene rearrangements at the level of FT1 including gene deletion and gene conversion events. The role of point mutations in MTX resistance was validated by gene editing strategies. We showed that cells with the FT1 $^{P555S/+}$ version (mutated in the transmembrane domain), FT1 $^{A430V/-}$ or FT1 $^{G129D/-, A121T/-}$ versions (mutated in the intracellular domain) were 5-fold, 38-fold and 135.6-fold more resistant to MTX compared to the control cells,

respectively. WT cells with DHFR-TS $^{E291K/+}$ (mutated in TS domain, near the active site) and DHFR-TS $^{T107I/+}$ (mutated in DHFR domain, near the MTX binding pocket) versions were 4.4-fold and 2.9-fold more resistant to MTX compared to the control cells, respectively. Dominant positive effects were highlighted for three mutations in DHFR-TS and PTR1 genes. Episomal transfections of a DHFR-TS ^{T107I}, PTR1 ^{A28T} or PTR1 ^{S253F} versions in a WT strain were 2.5-fold, 6.9-fold and 9.4-fold more resistant to MTX compared to WT version of the gene overexpressed, respectively. Genes never associated with folate metabolism coding for L-gulonolactone oxidase or for the hypothetical protein LmjF.17.1130 were mutated in specific mutants. Transfection of WT versions of these genes reverted resistance to MTX by 2- and 3-fold, respectively. A single Mut-seq screen and the sequencing of twenty MTX resistant clones has allowed to map all known genes involved in folate metabolism and has highlighted novel genes as well. Mut-seq is a powerful tool to further our understanding of one carbon metabolism in *Leishmania*.

Keywords RESISTANCE; METHOTREXATE; SEQUENCING; FOLATE; MUT-SEQ

P3-087: ANALYZING THE EMERGENCE OF PARASITISM WITHIN KINETOPLASTIDS: A POSSIBLE CORRELATION WITH INOSITOL PYROPHOSPHATES

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Inositol pyrophosphates (PP-IPs) are highly energetic metabolites involved in a wide range of cellular processes in eukaryotes. The most common PP-IPs – IP_7 and IP_8 – are synthesized through complementary pathways that involve IP6K and PP-IP5K kinases, respectively. Trypanosomatids parasites (e.g.: *Trypanosoma cruzi* and *Leishmania* spp.) have orthologous genes for IP6K, but the genes encoding to PP-IP5K are apparently absent. Curiously a free-living kinetoplastid organism – *Bodo saltans* – has a PP-IP5K homolog with 42% identity relative to human PP-IP5K. The goal of this study is to investigate whether the absence of PP-IP5K (and consequently IP_8) is mutually exclusive relative to parasitism within kinetoplastids. Thereunto, we carried out evolutionary analyzes to confirm the alleged loss of PP-IP5K in trypanosomatids. We reconstructed phylogenetic trees and got robust evidence that confirms the absence of PP-IP5K in all trypanosomatids but *Paratrypanosoma confusum*. Predictions of the tertiary structures point out that the catalytic domain of *P. confusum* PP-IP5K is naturally unstructured, which puts its function in check. Besides, we performed molecular dynamics assays with *B. saltans* PP-IP5K (BsPP-IP5K) and IP_7, which was used as substrate (together ATP) to synthesize IP_8. Our simulations suggest that BsPP-IP5K is fully capable of synthesizing IP_8. This way, we amplified the BsPP-IP5K gene and added to it the 3' and 5' UTR regions from Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH). Subsequently, we

cloned this construction into the pSP72-αHYGα vector (Marc Ouellette lab), generating a plasmid that provides episomal constitutive expression of BsPP-IP5K into *Leishmania braziliensis* (episomal knock-in). We are performing phenotypic characterization of the generated lineage, in addition to planning infection assays. Also, we intend to perform knock-out (KO) of the PP-IP5K from murine macrophages (host cells) and use them to measure the infection capacity of the *L. braziliensis* knock-in lineage. Our findings, although preliminary, suggest that the transition from a free-living to a parasitic lifestyle has resulted in the loss of PP-IP5K. This work will clarify if the PP-IPs have any relation with the parasitism developed within the kinetoplastids, contributing to unveiling new routes for the developing antileishmania therapies.

Keywords INOSITOL PYROPHOSPHATES; KINETOPLASTIDS; *Leishmania braziliensis*; EPISOMAL KNOCK-IN

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P3-088: IDENTIFICATION OF INOSITOL PYROPHOSPHATES TARGET PROTEINS IN *Leishmania braziliensis* – A PRELIMINARY APPROACH

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In model eukaryotes, inositol pyrophosphates (PP-IPs) – mainly IP_7 and IP_8 – are involved in a wide range of cellular processes. However, target proteins of PP-IPs, as well as their mechanisms of action, are still poorly understood. IP_7 and IP_8 are synthesized through complementary pathways that require the kinases IP6K and PP-IP5K, respectively. *Leishmania* spp have an ortholog of IP6K, but lack orthologs of PP-IP5K, meaning this parasite is unable to synthesize IP_8. Thus, the main objective of this work is to track and identify the main target proteins of IP_7, using *Leishmania braziliensis* as a model. To pursue this goal, we performed the cloning of recombinant *L. braziliensis* IP6K (LbrIP6K), as well as its N-terminal catalytic domain, and IP5K (which will be used as control) using the pET28a+ and pET32a+/pET32 Ek/LIC systems. After transforming these constructions into different *E. coli* strains (BL21-CodonPlus-(DE3)RP; BL21(DE3)pLysS; and BL21(DE3) ArcticExpress, we identified expression of these kinases (using pET32a+) after 2 h induction of 1 mM IPTG in BL21-CodonPlus-(DE3)RP and BL21(DE3) ArcticExpress strains. These proteins are being purified using affinity chromatography (Ni^{2+} columns), and they will be used to synthesize in vitro IP_7-labeled with γ-(Propargil)-imido on the β-phosphate moiety (IP_7-labeled). As a control, IP5K recombinant will be used to synthesize IP_6-labeled (which is a non-pyrophosphate). Alternatively, the N-terminal catalytic domain of IP6K from *Trypanosoma cruzi* [TcIP6K(ArgRIII)] is also being expressed and purified, and it could be used to synthesize IP_7-labeled in case we face problems with LbrIP6K. This approach will allow us to selectively conjugate biotin – via a 'click' chemistry reaction – to *L. braziliensis* proteins that have received the β-phosphate from IP_7-labeled. We will then isolate biotin-labeled proteins using streptavidin

immunoprecipitation and identify them using LC-MS/MS. Given pyrophosphorylation by PP-IPs is not enzymatic, our work may uncover new routes for drug development and, therefore, the effective treatment of leishmaniasis.

Keywords INOSITOL PYROPHOSPHATES; *Leishmania braziliensis*; RECOMBINANT PROTEINS; PYROPHOSPHORYLATION; MASS SPECTROMETRY

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P3-089: BIOCHEMICAL CHARACTERIZATION OF THE 23-KDA CYSTEINE PROTEASE, CP23E, FROM *Leishmania mexicana* EXOPROTEOME

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Protozoan parasites of the *Leishmania* genus are associated with a broad spectrum of diseases ranging from self-healing cutaneous lesions to lethal visceral consequences. These parasites release protein factors as adaptive mechanisms that contribute in the infection and maintenance processes of the genus. These protein factors are part of the exoproteome, which is defined as the set of total proteins released into the extracellular space by an organism under defined conditions. Among these protein factors, proteases are considered key factors in the parasite survival inside the mammalian host. Particularly, the cysteine proteases (CP) activity present in these parasites have been related with their virulence, becoming interesting the study of this protease family in *L. mexicana* exoproteome. Previous results suggest the possible presence of a 23KDa CP (CP23E), in the promastigote and amastigote forms of *L. mexicana* exoproteome, as a potential candidate for further studies, since its proteolytic activity could be related to the parasite virulence. Hence, our aim focusses on the purification and characterization of the CP23E from *L. mexicana* promastigotes exoproteome. CP23E proteolytic activity was analyzed through gelatin-SDS-PAGE, incubating the exoproteome *of L. mexicana* axenic promastigotes for 24 hours in an appropriate medium for the development of CP activity. Preliminary results allowed us to establish CP23E optimum activity at 50°C and pH 6.0, pH range from 3.0 to 8.0 and temperatures between 4°C to 60°C. Therefore, we conclude that the protease shows to be a thermostable neutral cysteine protease.

Keywords *Leishmania mexicana*; EXOPROTEOME, CP23E; pH; TTEMPERATUR

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P3-090: COMPARATIVE STUDY OF THE TOTAL PROTEOLITIC ACTIVITY OF *Leishmania mexicana* PROMASTIGOTES AND AMASTIGOTES EXOPROTEOME

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Leishmania are protozoan parasites with a complex life-cycle, involving several developmental forms. These forms represent an adaptation to the changing environmental conditions encountered by the parasites within their two hosts: the mammalian host, to which they are pathogenic, and the sandfly insect vector. In the sandfly, *Leishmania* replicate as extracellular flagellated and three main forms can be distinguished. Promastigotes, which are multiplicative but not mammalian-infective resides primarily in the insect's alimentary tract. Procyclic promastigotes, there are present in the insect's midgut; non-dividing, but mammalian-infective and metacyclic promastigotes, also infective, in the thoracic midgut and proboscis of the sandfly. The metacyclic promastigotes, when inoculated into a mammalian host through a sandfly bite, differentiate into the intracellular aflagellate amastigote form. During infection, the parasite deploys adaptive mechanisms that allow it to survive the changes imposed by the different environments where they develop. In some cases, the mechanisms of invasion involve the export of proteins factors into the cytosol of the host cell which are part of the exoproteoma (EP). Several exported proteases have been linked as virulence factors. However, only a few of the proteases present in the EP have been identified and characterized. In this opportunity our aim focuses on the comparative study of the proteolytic activity present in the EP of the main morphological stages of *Leishmania mexicana*. Axenic promastigotes, in different growth phases and amastigotes of *L. mexicana,* were incubated for 24h at room temperature and 32-35°C, respectively, in RPMI medium, in order to allow EP release. Subsequently, the supernatants

were recovered by centrifugation at 15,000×g for 30 min and analyzed by zymograms on polyacrylamide gels copolymerized with 0.1% gelatin. The gels were incubated at different pHs, under selective conditions for the development of the activities of the different protease families, in the presence and absence of specific inhibitors. The zymograms revealed a differential pattern of proteolytic activity present in the EP of the three morphotypes studied. On the other hand, under our study conditions, the differential activity of the main protease families reported in this parasite could be evidenced. The study and identification of the function of the detected proteolytic activities will allow us to know their participation in the maintenance and survival mechanisms of the parasite in the different stages of its life cycle and its possible use as a chemotherapeutic target.

Keywords *Leishmania mexicana*; EXOPROTEOME; PROMASTIGOTE; AMASTIGOTE

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P3-091: SECRETED *Leishmania* CASEIN KINASE 1 BINDS TO AND PHOSPHORYLATES HOST PROTEINS

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Host-pathogen interaction is a pivotal phenomenon that determines the final outcome of the infection. Upon entering the host, pathogens secrete an array of molecules to modulate its environment and ensure its survival and proliferation. Casein kinase 1.2 (CK1.2) is a signalling kinase secreted by *Leishmania* via exosomes into the host cell upon infection. This kinase is a validated drug target due to its indispensable role in parasite survival. Its sequence shows extreme conservation within *Leishmania* species and has high similarity with mammalian CK1s. We thus hypothesize that the secreted pool of L-CK1.2 might play an important role in subverting host signalling pathways by phosphorylating proteins within the host cell. This hypothesis was first tested under *in-vitro* conditions. To identify host-binding partners of L-CK1.2, we performed an immuno-precipitation by incubating bone marrow-derived macrophage lysates (BMDM) with or without recombinant L-CK1.2-V5. We identified 239 binding partners, among which 52 were proteins known to interact with mammalian CK1s. To identify *bona fide* L-CK1.2 substrates, we developed a novel substrate screen, combining phosphatase treatment, *in vitro* kinase assay and Stable Isotope Labelling with Amino acids in Cell (SILAC) culture-based mass spectrometry, to compare the phospho-proteome of pulse-heat inactivated THP-1 lysate in presence or absence of recombinant L-CK1.2. We found 225 host substrates, belonging to various protein classes including chaperone or

transcription factors. This finding indicates that L-CK1.2, like mammalian CK1s, may regulate many biological processes in the host cell. Among the host substrates and binding partners identified in the two screens, we shortlisted 12 candidates to investigate their abundance in *L. donovani* infected versus uninfected BMDMs by Western blot analysis. According to our preliminary findings, a subset of proteins showed significant alteration in their levels and interestingly, these change in abundance appear to be *Leishmania* species-dependent. To confirm our hypothesis regarding the impact of secreted L-CK1.2 on the host cell and to validate our *in vitro* L-CK1.2 interactome and substratome results in a cellular model, we are currently performing transcriptomic and phosphoproteomic analyses of *L. donovani* infected or uninfected BMDMs, treated with or without the L-CK1 inhibitor D4476. Our findings will not only provide an insight into host pathways targeted by the secreted L-CK1.2 pool, but also provide novel information regarding the host signalling status following *L. donovani* infection.

Keywords CASEIN KINASE 1; SECRETED PROTEINS; CELL SIGNALLING; HOST-*Leishmania* INTERACTIONS; OMICS

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P3-092: STUDIES ON THE MITOCHONDRIAL PYRUVATE CARRIER IN *Leishmania (Viannia) braziliensis*

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Leishmaniasis is a neglected tropical disease caused by different parasites of *Leishmania* genus. *Leishmania (Viannia) braziliensis* is one of the main etiological agents of tegumentary leishmaniasis in the Americas. In most eukaryotic cells, pyruvate plays a central metabolic role by interconnecting glucose metabolism, the tricarboxylic acid cycle (TCA) and the oxidative phosphorylation. In trypanosomatids, the first seven steps of glycolysis are compartmentalized in a peroxisome-like organelle called the glycosome. Pyruvate is produced from phosphoenolpyruvate by the cytoplasmic pyruvate kinase, and can be converted to alanine or transported to the mitochondria by the mitochondrial pyruvate carrier (MPC). In mitochondria, pyruvate will be metabolized via TCA cycle, providing reducing potential for oxidative phosphorylation. In several models, inhibition of MPC promoted a metabolic reprogramming involving the use of alternative substrates for maintenance of cellular homeostasis. Considering the importance of pyruvate metabolism for cell physiology, here we investigated mitochondrial pyruvate transport in *L. braziliensis*. In the *Leishmania* spp. genome, 2 putative MPCs are encoded. These are expressed by several species of *Leishmania*, observed by proteome database (TriTrypDB). Exposure of *L. braziliensis* promastigotes to the classical MPC inhibitor UK5099 caused no changes in cellular viability at low concentrations, but increased viability at high concentrations, without changes in proliferation. However, when the promastigotes were grown in the presence of UK5099, higher proliferation was observed during the logarithmic phase, time of great replication and metabolic activity.

Respirometry experiments on intact parasites treated with UK5099 caused no apparent changes in mitochondrial metabolism. However, experiments using parasites with chemically permeabilized plasmatic membranes revealed that parasites treated with the inhibitor consumed less oxygen in a pyruvate/malate and ADP dependent manner. When parasites were challenged with minimal media containing glucose as the sole carbon source, MPC inhibition by UK5099 treatment significantly reduced parasite viability, suggesting that this parasite may display alternative routes for the entry of carbons in the mitochondria. Parasite's genome displays putative MPC1 and 2, that are also observed as peptides according to proteomic data (TriTrypDB). Additionally, functional analysis demonstrated that oxygen consumption was inhibited by UK5099 in a pyruvate/malate/ADP dependent manner and proliferation was affected by the inhibitor during time course analysis. The results obtained so far indicated that *Leishmania* spp. undergoes metabolic adaptations during mitochondrial pyruvate transport inhibition, revealing a metabolic plasticity.

Keywords *Leishmania*; PARASITIC BIOCHEMISTRY; ENERGETIC METABOLISM; MPC; PYRUVATE

Financing POM/FIOCRUZ, PAEF/FIOCRUZ, CNPq, FAPERJ

P3-093: CHARACTERIZATION OF THE SPHINGOSINE KINASE (SK) ACTIVITY OF *Leishmania mexicana* AMASTIGOTES

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Leishmaniasis are a set of vector-borne diseases, caused by protozoan parasites from the genus *Leishmania,* that affect about 12 million people in tropical and sub-tropical areas of the planet. *Leishmania* life cycle alternates between two stages: the promastigote, an elongated and flagellated form present in phlebotomine sand flies, and the amastigote, an intracellular non motile form, responsible for maintaining the infection in the mammalian host. It has been demonstrated that the sphingolipid metabolism plays an important role in *Leishmania* survival, differentiation, morphology and ability to cause pathology. Sphingosine kinases (SKs) are important enzymes in this metabolic pathway responsible for the conversion of sphingosine into sphingosine-1-phosphate (S1P), a messenger involved in the regulation of important cellular processes in mammals. In 2013 it was identified in *Leishmania major* a gene encoding an enzyme homologous to mammalian SKs, which activity is essential for the elimination of toxic metabolites, survival under stressful conditions, and establishment of disease in mice by the parasite. Our group has characterized biochemically the SK activity from *Leishmania mexicana* promastigotes, and demonstrated the existence of two polypeptides with SK activity in this stage. In this work we set up to study the SK activity of *L. mexicana* amastigotes and to compare it with that of promastigotes from different culture phases. We demonstrated the existence of SK activity in amastigote-like forms of *L. mexicana* cultured for three days under axenic conditions. The compound DL-threo-dihydrosphingosine (DHS), a general inhibitor of mammalian SKs, inhibited only 37% of the activity. The SK activity of amastigotes-like forms is 1.34 times lower than that of logarithmic phase (mid-log) promastigotes, and 2.48 times lower than that of stationary (sta) phase promastigotes.

Western blot analysis, using a polyclonal IgY anti-human SK2 antibody, showed the presence of two bands, of 94 kDa and 45 KDa, whose relative intensities are: amastigotes > mid-log promastigotes > sta promastigotes. The immunofluorescent labelling and confocal microscopy analysis of amastigotes recently extracted from experimental lesions, with antibodies directed against the human SK2 enzyme, showed a cytosolic distribution, with a trend to accumulate in peripheral regions and certain organelles not yet identified.

Keywords *Leishmania*; AMASTIGOTE; SPHINGOSINE KINASE

P3-095: FUNCTIONAL CHARACTERIZATION OF PUTATIVE LONG NON-CODING RNAS DIFFERENTIALLY EXPRESSED DURING *Leishmania (Viannia) braziliensis* LIFE CYCLE

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Leishmania (Viannia) braziliensis is an important causative agent of cutaneous and mucocutaneous leishmaniasis in Americas. During its life cycle, this parasite colonizes two different hosts facing dramatic changes in physiological conditions which requires a fast and dynamic gene expression modulation in order to survive. The genetic organization of *Leishmania*, where unrelated genes are transcribed by RNA Polymerase II as polycistronic units in the absence of canonical promoters, suggests that the regulation of gene expression in these organisms results mainly from other processes rather than transcription itself. Non-protein coding RNAs (ncRNAs) have been identified in different trypanosomatids parasites, including *L. braziliensis*. In the latter, the transcriptome of the main morphologies across the *Leishmania* lifecycle (procyclic promastigotes, metacyclic promastigotes and axenic amastigotes) were compared and revealed the presence of 11,372 putative ncRNAs of which at least 295 were differentially expressed in all three stages. Using CRISPR/Cas9, we are investigating the functional role of these elements in *L. braziliensis*. Up to date, 14 ncRNAs (including short and long ncRNAS) were successfully knocked out from *L. braziliensis* M2903 genome by our group and phenotypically screened for phenotypic alterations. Herein, we present the results obtained for three potential long ncRNAs (lncRNAs) each of them enrolled in different phenotypes of *L. braziliensis.* The fitness of each knocked out line was compared to the parental wild-type line (WT) in experiments mimicking important points of *Leishmania* life cycle such as

parasite multiplication, survival to oxidative and nutritional stresses, metacyclogenesis and infectivity. We found that deletion of lncRNA66 led to a significant reduction in parasite growth as promastigotes whereas the deletion of lncRNA31 increased the doubling time of axenic amastigotes from 8.7 hours (WT) to 11.6 hours. Deletion of lncRNA52 resulted in a lower percentage of metacyclic parasites recovered after Ficoll enrichment, suggesting that this lncRNA may be enrolled in metacyclogenesis in *L. braziliensis*. Characterization of these transcripts by northern blotting, determination of ncRNA extremities and posttranscriptional processing (presence or absence of CAP and PolyA tail) by RNA circularization coupled with sequencing, and identification of possible ligands using a S1M tag-mediated pull down are currently ongoing to better understand the biogenesis and role of these putative regulatory ncRNAs in the parasite. Our results will help to understand the regulation of *Leishmania* gene expression and may result in the discovery of ncRNAs enrolled in parasite fitness and pathways essentials for parasite survival.

Keywords *Leishmania braziliensis*; NON-CODING RNA; GENE EXPRESSION; CRISPR/CAS9; FITNESS

Financing FAPESP, CAPES, CNPq and JCPiL

P3-096: LEISHSIPHER: OXIMETRY-BASED CELL ENERGY PHENOTYPE SCREENING TO CHARACTERIZE *Leishmania* PARASITES

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Leishmania must avoid reactive oxidants derived from oxygen (ROS) and nitrogen (RNS) exogenously to establish infection in the host. Following drug exposure, there is an overproduction of ROS in *Leishmania* mitochondria that acts as a downstream effector mechanism, leading to parasite's death. Measuring this activity could provide clues for screening drug candidates for the treatment of leishmaniasis. More than that, real-time axenic oximetry could be applied to rapidly identify drug resistant parasites, field/clinical isolates and quiescent strains based on their particular features on mitochondrial respiration. To test this hypothesis, here we present for the first time the use of Lucid Scientific RESIPHER system that allows to accurately measure oxygen consumption directly in *Leishmania* axenic cultures in real time compatible with long-term (days) measurements. Optical oxygen sensors provide a high reading sensitivity without disturbing the culture. The oxygen concentration gradient in the medium is measured as a direct result of parasite oxygen consumption rate (OCR). The reading is achieved by continuously scanning the probes vertically above the culture to measure the gradient and, using signal

processing algorithms to convert the concentration readings into OCR. The RESIPHER device is portable, easy to handle and allows remote monitoring from a smartphone App. Given the great advantages of this equipment, we used it to study the effect of different drugs on the OCR of laboratory adapted and *Leishmania* field strains. For this purpose, we used laboratory-selected resistant strains, in the presence of antimony, miltefosine or amphotericin B and sensitive strains (LdiWT) in the presence and absence of the 3 above mentioned drugs. We also evaluated the O_2 flux in antimony-resistant and antimony-sensitive field strains. As well, we evaluated the effect of rotenone (a mitochondrial NADH:ubiquinone reductase inhibitor) in the different conditions. Lastly, we monitored the oxygen exchange during *Leishmania*-macrophage infection. All these conditions, we analyzed by adding the culture in a 96-well plate, to which we attached the RESIPHER device and incubated them at 25 °C for 5 days. To optimize the use of RESIPHER, we first identified $5x10^6$ parasites/mL as the optimal concentration to perform the experiments. Secondly, we found that resistant strains show less O_2 consumption than sensitive strains. In addition, we observed that when we added at 24 h the inhibitor -rotenone- there is a decrease in oxygen flux in all the strains. The results shown give us an indication that culture monitoring using RESIPHER could be useful in drug screening for leishmania treatment, drug dose-response or experimental infection assays with high reproducibility between experiments due to its ease of use. The system can be applied to easily differentiate between aerobic and quiescent parasites being compatible with non-adherent motile parasites.

Keywords RESIPHER; DRUG RESISTANCE; *Leishmania*; OXYGEN FLUX; ROTENONE

P3-097: CHARACTERIZATION OF CALCINEURIN IN DIFFERENT INFECTIVE FORMS OF *Leishmania amazonensis*

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Leishmaniasis is a neglected tropical disease, caused by protozoan parasites called *Leishmania*, which are transmitted by sandflies during the blood meal. These parasites infect different phagocyte immune cells by infective forms of *Leishmania*, called metacyclic promastigotes (released during the blood meal) or amastigotes (main form in the mammalian infection establishment), which present important differences related to morphology, molecular mechanisms and specific functional proteins, such as parasite enzymes, depending on the biological cycle. Calcineurin (CaN) is a heterodimer Ca^{2+} dependent phosphatase involved in different cellular process related to thermo-tolerance and adaptation to oxidative stress, important in these cycle of parasites due to change in ph and temperature mainly. In this study, we examined *Leishmania amazonensis* CaN from the two different infective forms (promastigotes and amastigotes) based in the activity, expression and localization of this important parasite enzyme. Promastigotes (stationary phase culture containing metacyclic forms) and amastigotes were treated with CaN specific inhibitors (Cyclosporin A, FK506) and parasite viability was measured by MTT assay. To analyze CaN expression, total amount of protein extract from the different parasite forms were analyzed by western blot by using specific antibodies against CaN. For subcellular localization, we performed two experimental approaches: Western blot following cellular parasite fractionation (cytoplasm, plasma-membrane, nuclear and cytoskeleton fractions), and immunofluorescence

microscopy (including confocal microscopy) to visualize intracellular fluorescence staining. *Leishmania* promastigote and amastigote presents different susceptibility to CaN inhibitors. When both forms are treated with 80 µM of FK506, more than 90% of both infective forms are viable. However, promastigotes are more susceptible to CsA, diminishing parasite viability to 60% (40 µM) and 35% at 80 µM, comparing to amastigotes that are more resistant with almost 80% still viable at 80 µM of treatment. More important, CaN is differentially expressed in both forms, presenting differences in its localization depending on the infective form, which could be involved in the different functional aspects of this important enzyme during the biological cycle of *Leishmania*.

Keywords AMASTIGOTES; CALCINEURIN; CYCLOSPORIN; FK506; *Leishmania amazonensis*; PROMASTIGOTES

Financing FAPESP; CNPq; CAPES

P3-098: GENOMES OF *Leishmania (Viannia)* STRAINS FROM BRAZILIAN AMAZON AND THEIR PUTATIVE NATURAL HYBRIDS

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Brazil accounts for more than 40% of Tegumentary Leishmaniasis (TL) cases registered in the American Continent. Most cases of TL in Brazil are caused by parasites of the subgenus *L.* (*Viannia*), and are concentrated in the North Region, the Amazon biome. *Leishmania* (*V.*) *braziliensis* is the most dispersed specie, presented in different endemic area. Additionally, five other species have already been identified as human pathogens in Brazilian Amazon: *L.* (*V.*) *guyanensis*, *L.* (*V.*) *shawi*, *L.* (*V.*) *lainsoni*, *L.* (*V.*) *naiffi* and *L.* (*V.*) *lindenbergi.* Recently *L.* (*V.*) *utingensis* has been also detected, by molecular tests only, causing human disease in Roraima (North of the Brazilian Amazon). Sympatry of *Leishmania* species is frequently reported in the North of Brazil, eventually sharing the vertebrate and invertebrate hosts, ultimately favoring the occurrence of hybrids. Putative natural hybrid strains of *L.* (*Viannia*) were reported in the last decades, and they are likely to occur by a process of genetic recombination during invertebrate stage. The *Leishmania* genomics has unfold novel paradigm during the last decades, as well as new perspectives on aneuploid mosaicism and gene sinteny. In this study genomes of *L.* (*Viannia*) strains from the Brazilian Amazon were sequenced using the Sequencing by Synthesis (SBS) method on an Illumina platform. For the *de novo* genome assemblies A5-MISEQ pipeline (https://sourceforge.net/projects/ngopt/) was used. In order to reduce gaps and redundancies in scaffolds assembled, REDUNDANS software (https://github.com/lpryszcz/redundans) was used. The chromosome level contiguation and gene annotation was performed using

COMPANION software (https://github.com/sanger-pathogens/companion), with *L*. (*V*.) *braziliensis* (M2904) genome, available on TriTrypDB, as reference. Here, the genomes of 13 *L*. (*V*iannia) strains were sequenced. This study brings the unprecedented *de novo* genomes for *L*. (*V*.) *utingensis* and *L*. (*V*.) *lindenbergi* species, and three strains of putative natural hybrids characterized by the MLEE method: *L*. (*V*.) *lainsoni* / *L*. (*V*.) *naiffi*, *L*. (*V*.) *braziliensis* / *L*. (*V*.) *naiffi* and *L*. (*V*.) *braziliensis* / *L*. (*V*.) *guyanensis*. The genome was also sequenced for *L*. (*V*.) *braziliensis*, *L*. (*V*.) *guyanensis*, *L*. (*V*.) *shawi*, *L*. (*V*.) *lainsoni* and *L*. (*V*.) *naiffi* species, representing newly sequenced strains. The number of mounted scaffolds varied between 1,456 (N50=49,706pb) and 2,964 (N50=22,164pb) indicating the good quality of assemblies. The number of annotated genes of our sequenced genomes varied between 8,106 and 8,191 genes. Our data will allow analysis of gene and genomic structure predictions, SNPs, polymorphisms, and dissimilarity in the putative natural hybrids sequenced. The investigation of genetic composition is vital to understand the phylogenetic relationship between these species and their genomic-based adaptation mechanisms. These newly released *L*. (*Viannia*) genomes are of particular interest for genetic, evolutionary, and physiological understanding of *Leishmania* biological issues.

Keywords LEISHMANIASIS; GENOMIC; NATURAL HYBRIDS; BRAZILIAN AMAZON

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P3-099: COMPARISON OF GENE EXPRESSION PROFILE IN HUMAN MACROPHAGES INFECTED BY *Leishmania* (*Viannia*) *braziliensis* CONTAINING VIRAL ENDOSYMBIONT LRV1

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The parasite *Leishmania* (*Viannia*) *braziliensis* is widely distributed in Brazil, being one of the main species associated with human cases of cutaneous leishmaniasis (TL). In addition to cutaneous leishmaniasis (CL), *L. braziliensis* is closely related to the development of mucosal leishmaniasis (ML) and atypical cutaneous lesions. The mechanisms underlying the pathogenesis of TL are not yet fully understood, but it is known that factors related to the host and the parasite act synergistically and relevantly to direct the response to infection. In the host, the macrophage is the central relationship with the parasite, with a fundamental role in the defense of the organism due to its ability to destroy intracellular *Leishmania* and present antigens. In the parasite, some intrinsic factors related to the species or even the analyzed strain are fundamental for the outcome of the disease, such as the presence of Leishmania RNA Virus 1 (LRV1), a virus that parasitizes some *Leishmania* (*Viannia*) species and triggers a cascade of signals that lead to a more severe TL phenotype, such as ML. One of the strategies for understanding factors associated with the immune response generated after

Leishmania/host interaction is the analysis of cellular patterns after infection. Therefore, we analyzed the gene expression profile of macrophages derived from human monocytes obtained from healthy donors infected with *L. braziliensis* LRV1 positive (LbLRV1+) and negative (LbLRV1-). We used a microarray platform (Applied Biosystems® Microarray) that simultaneously analyzes the expression of more than 20,000 human genes. The expression values represent the average obtained from the comparative analysis of cells from three independent donors. The results show differential expression of several genes related to signaling pathways mediated by Interferons type I and II, the Nod Like receptor signaling pathway and adipogenesis pathway, with overexpression or under expression of different genes in cells infected with the strain LbLRV1+ compared to the LbLRV1- strain and the negative control. The data suggest the activation of signaling pathways associated with the presence of LRV1 already reported in the literature, validating the applied methodology, and includes genes from pathways not yet reported. The study shows for the first time the activation of interferon-mediated pathways and the regulation of the adipogenesis signaling pathway, under these conditions. The use of newly isolated human primary cells presents genotypic and phenotypic characteristics closer to the tissue of origin, and it is advantageous to use macrophages derived from human monocytes to investigate gene expression. Although, the results of differentially expressed genes on the microarray analyzes will be confirmed by qPCR and by the quantification of proteins related to these genes. The initial results reinforce the role of LRV1 in directing the post-infection host immune response and its analysis in patients with TL may be useful in screening patients to assess the course of the disease.

Keywords *Leishmania braziliensis*; *Leishmania* RNA VÍRUS 1; LRV1; GENE EXPRESSION

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P3-100: CYSTEINE PROTEASE ACTIVITY IN THE EXOPROTEOME OF *Leishmania mexicana:* IT`S ROLE IN THE PARASITE VIRULENCE

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Leishmania sp. during transmission deploys adaptive strategies that allows it to survive in the different environments of development. Linked to the adaptive mechanism have been reported protein factors released to the extracellular environment, under determined conditions, named exoproteome. The exoproteome contains proteases, as parts of the exodegradome which have been considered important therapeutic targets given their demonstrated participation in the processes of infection, replication and development of the parasite. Among the proteases present in the exoproteome of *Leishmania mexicana* we have studied the cysteine proteases as candidates related to the parasite virulence. The exoproteome of amastigotes and those from attenuated and virulent promastigotes was compared using electrophoretic techniques such as SDS-PAGE and zymograms. It was found a dissimilar protein pattern as well as a differential cysteine protease activity between the exoproteomes of both morphotypes and a decrease in the activity of attenuated parasites compared to their virulent forms. These results points to the cysteine proteases as good therapeutic targets and a direct relationship between parasite virulence and these proteases.

Keywords *Leishmania*; PROTEASES; AMASTIGOTES; PROMASTIGOTES; EXOPROTEOME

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P3-101: INVESTIGATING HUB GENES IN CUTANEOUS LEISHMANIASIS CAUSED BY *Leishmania tropica* THROUGH LONG NON-CODING RNA AND MRNA CO-EXPRESSION NETWORK MODELING

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Recently, considerable attention is directed towards the long non-coding RNAs (lncRNAs) as one of the pivotal epigenetic regulators of gene expression and disease progression. Emerging evidence indicated that lncRNAs play a vital role in the biology of skin and the pathology of cutaneous diseases. Cutaneous leishmaniasis (CL) is a parasitic skin infection considered the most prevalent form of leishmaniasis and remained a health challenge in many countries. However, the role of lncRNAs and their pattern regulation is a new avenue remained to be explored in the CL diseases. In this regard, we investigated the lncRNAs expression profile and explored the hub lncRNAs in CL diseases caused by *Leishmania tropica*, as one of the main causative agent of CL in Iran. Here, the raw RNA-Seq data of our previous study composed of skin lesions from patients with ulcerative CL (UCL) and non-ulcerative CL (NUCL) caused by *L. tropica* and skin samples of healthy volunteers were used and re-annotated by gene biotype. The R package of Weighted Gene Co-expression Network Analysis (WGCNA) was employed to make lncRNAs and protein-coding gene co-expression network and find significant modules. Selecting hub genes and functional enrichment analysis associated with the modules were performed, as well. Finally, the LncTar database was utilized to predict the potential interactions of the selected coding-non-coding hub genes. WGCNA found that the turquoise and brown modules were significantly correlated with our studied clinical traits (UCL and NUCL) in the seven identified modules. Both turquoise and brown modules were enriched mostly in Interferon gamma signaling, cytokine signaling in the immune system, and interleukin-12 family signaling pathways. The top 20 genes were selected as hub genes

in each significant module. In subsequent analysis, three co-expressed lncRNAs/protein-coding genes were determined among the selected hub genes list in the turquoise module through LncTar-based prediction, namely, IL21R-AS1/PDAP1, IL21R-AS1/ OR2I1P, and MBNL1-AS1-1/KCNA3 genes. In the brown module, the LncTar-based co-expressed hub genes were ALDH1L1-AS1/TIMP4 and ALDH1L1-AS1/PSG2. Our findings shed light on the integrated regulatory networks (lncRNA- mRNA) in the development of CL (UCL/NUCL) disease caused by *L. tropica*. Moreover, the selected hub genes could be promising for diagnostic and therapeutic purposes for CL. However, further investigation is crucial to confirm the functional importance of the predicted lncRNA genes.

Keywords *Leishmania tropica*; CUTANEOUS LEISHMANIASIS; LONG NONCODING RNA; WGCNA

P3-102.1: ANALYTICAL AND DIAGNOSTIC PERFORMANCE OF THE MINIEXON PCR-RFLP FOR DIAGNOSIS AND TYPING OF *Leishmania* spp. FROM NON-INVASIVE CLINICAL SAMPLES

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WHO recommends species identification along with a confirmed diagnosis in cutaneous leishmaniasis patients to facilitate treatment planning, evaluate prognosis, and to compile epidemiological information. Miniexon gene amplification allows the identification of species *L. (L.) amazonensis, L. (L.) mexicana*, and *L. (L.) chagasi*, as well as the species *L. (V.) braziliensis* of the Leishmania Viannia complex by RFLP-HaeIII. However, analytical performance has not been evaluated, and few studies have determined the diagnostic performance of this methodology. Two independent analysts determined the detection limit and repeatability of the assay (analytical sensitivity). The selectivity, exclusivity, and inclusivity of the assay were analyzed to evaluate the analytical specificity. From non-invasive clinical samples, diagnostic performance was evaluated. This included assessing diagnostic sensitivity and specificity, as well as accuracy, false positive and false negative rates. Finally, infecting species were identified in the clinical samples with a positive diagnosis. The detection limit of the technique was 0.5 ng/ul of DNA, and the Kappa concordance index from the repeatability assay was 1. The technique only amplified the genomic sequence of interest (selectivity of the assay) in positive samples, no cross-reaction with microorganisms of the skin microbiota was observed (exclusivity of the assay) and amplification of the miniexon gene was achieved in all species circulating in Colombia (inclusivity of the assay). The diagnostic performance of the technique showed sensitivity and specificity values of 85% and 100%, respectively. The accuracy of the test was 92.52%, the false positive rate was 0 and the false negative rate was 0.15. In 49 of 74 positive

clinical samples, *L. braziliensis was identified as the infecting species* (66.2%), and the remaining (25 samples) corresponded to the *L. panamensis / L. guyanensis* complex (33.8%). The diagnostic performance of the technique showed sensitivity and specificity values of 85% and 100%, respectively. The results obtained allow an adequate diagnosis of leishmaniasis and classification of Leishmania spp. species, from non-invasive clinical samples.

Keywords CUTANEOUS LEISHMANIASIS; DIAGNOSIS; LEISHMANIA; SENSITIVITY AND SPECIFICITY

P4-048: DYNAMIC INTERACTOME CHANGES IN SUSCEPTIBLE AND RESISTANT MICE-DERIVED BMdMs UPON *Leishmania major* INFECTION

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The *Leishmania* infection outcome depends largely on parasite pathogenicity and virulence but also on the activation status and genetic background of host macrophages. In an attempt to elucidate how the host genetic background differences could determine the outcome of pathogen infection, we analyzed two different mouse strains with contrasted behavior in response to parasite infection and compared transcriptomic signatures of susceptible (Balb/c) and resistant (C57Bl/6) bone marrow derived macrophages (BMdMs) in response to *Leishmania major* infection. We first compared the M-CSF-induced transcriptomic profiles in monocytes derived from both mice strains. We next measured the transcriptional signatures of resistant and susceptible BMdMs at different time points after infection with promastigotes of the protozoan parasite *L. major*, and for each mouse strain identified dynamically altered genes by comparing the time series against the respective control time series with the tool MaSigPro. In order to identify network changes, we weighted the dynamically altered genes according to their deviation from the control time series, mapped these weights to a large protein-protein interaction network and performed network propagation with the NetCore tool. Our analysis revealed significant differences in 203

genes that are differentially expressed (DEG) in uninfected macrophages derived from both Balb/c and C57Bl/6 mice. Among these DEGs we find cathepsinE, β-catenin, Arg2 and Cxcl14 up-regulated in M-CSF-differentiated Balb/c macrophages and c, the macrophage-secreted protein CD5l and two E3 ligase enzymes up-regulated in M-CSF-differentiated C57Bl/6 macrophages. Moreover, different type I interferon-stimulated genes are differentially regulated between the two mice derived macrophages. These host differences could partly explain the different transcriptomic profiles observed in response to infection in Balb/c and C57Bl/6 derived macrophages. The differences are affecting, among others, the immune response as infected BMdMs reveal a repressed immune response in Balb/c BMdMs, a result confirmed by RT-PCR experiments performed on a selected set of genes. The network propagation procedure allowed us to judge differences in the response of the two mouse strains at the network level. In particular, the different networks emphasized the importance of metabolic pathways. It points out with no surprise the metabolism of carbohydrates, lipids and arginine an amino-acid necessary for multiple immune functions, including microbicidal nitric oxide (NO) production. A coordinated expression and sustained activation of nitric oxide synthase (iNOS) and arginine-succinate synthase (Ass1), validate by RT-PCR experiment, is observed in infected C57Bl/6 BMdMs that may result in the recycling of citrulline to supply the depleted intracellular arginine and fuel the NO production allowing the control of the parasite. The transferrin receptor and different soluble cytoplasmic glutathione-S-transferases, as well as peroxiredoxin (PRDX1) and the multidrug resistance protein 1 (MRP1) are also present in the module of infected C57Bl/6 macrophages suggesting a rigorous control of intracellular NO. In summary, we demonstrate that the host gene expression determines to a large degree mouse strain response to *L. major* infection, and that gene expression analysis in combination with network propagation can be used to identify dynamically altered, mouse strain-specific networks that hold mechanistic information about these different infection responses.

Keywords Balb/c; C57Bl/6; MPR1; NITRIC OXIDE; iNOS

P4-049: DISSECTING THE PROTEOMIC AND NUCLEIC-ACIDS CONTENT OF *Leishmania infantum* FIELD STRAINS AND THEIR EXTRACELLULAR VESICLES USING A MULTILAYER OMICS APPROACH

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Extracellular vesicles (EVs) have a diverse content including proteins, nucleic acids, lipids, virulence factors and transcription factors. In previous work, our laboratory demonstrated that the protein content of EVs is characteristic of the resistance (R) profile of different strains of *Leishmania infantum* (WT, R-Sb, R-MTF, R-AmB) through the presence of resistance-associated markers in EVs. Moreover, we showed that EVs are enriched in resistance genes that impacts both transmission of drug resistance genes and *Leishmania* biology. Given the critical need to detect drug-resistant strains, this project focuses on the discovery and functional characterization of unique/enriched proteins and nucleic acids released within *Leishmania* EVs that can be used as biomarkers. Eleven *Leishmania infantum* strains isolated from naturally infected dogs underwent phenotypic characterization (growth assessment, antimony resistance profiling in both promastigotes and amastigotes, as well as their infection capacity). Whole genome sequencing was performed by Illumina NovaSeq 6000 sequencer to detect CNVs and SNPs to identify genetic features that correlate with the

fitness of the strains. EVs were isolated by successive filtrations and two ultracentrifugations of 1h at 100,000xg. They were characterized according to size, quantity, purity, and integrity by nanoparticle tracking analysis, microBCA and transmission electron microscopy. We evaluated the transcriptomic and proteomic content of EVs, to better understand the role of small vesicles in the biology of *L. infantum* and to identify unique/enriched biomarkers of drug resistance in EVs. Briefly, transcriptomic profiling was performed by Illumina NovaSeq 6000 to identify differentially abundant transcripts between R and susceptible (S) parasites and EVs. Shotgun proteomics was also done by LC-MS/MS, functional annotations, signal peptide prediction were obtained, and differential protein abundance was compared between the R, S parasites, and their respective EVs. Phenotypic characterization led to a clear separation of the resistance profiles of the strains based on their EC_{50} value against antimony, of both promastigotes and amastigotes (S; n=4 and R; n=7). There is also a correlation between the resistance profiles and the genomic content of the parasites based on different chromosome ploidy associated with drug-resistance pathways. Whole parasite proteomics revealed 10 significantly up-regulated proteins and 11 proteins unique to the R-parasites. Proteomics of the EVs revealed 28 significantly up-regulated proteins and 28 proteins unique to R-EVs (released by R-parasites). Five mainly proteins were selected for a functional validation in *L. infantum* WT based on their enrichment in R-parasites and R-EVs; hypothetical protein, nodulin-like, malate dehydrogenase, putative ama1, and ecotin-like. Their resistance profiles against antimony revealed an increase between 1.3 to 3.3-fold change when compared to the mock-transfected *Leishmania* line. These multiomic analyses demonstrated differential enrichment associated with drug-resistant potential biomarker in parasites, which were reflected in their respective EVs. This project will provide novel, important insights in the composition and functions of EVs, as well as how the different biomolecules get packed in EVs depending on the genetic background of the releasing cell.

Keywords *Leishmania infantum*; DRUG RESISTANCE; PROTEOMIC; EXTRACELLULAR VESICLES; BIOMARKERS

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P4-050: THE ROLE OF PENTRAXINS IN *Leishmania* INTERACTION WITH PERMISSIVE SAND FLY MIDGUTS

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A crucial step in leishmaniasis elimination is understanding and preventing transmission of the parasite by sand flies. Attachment of nectomonad stage parasites to the midgut of the sand fly is an essential step in the *Leishmania* lifecycle. This interaction prevents the removal of the parasites when the blood meal remnants are defecated. The components involved in this attachment have been best characterised for the restrictive vector *Phlebotomus papatasi,* with *Leishmania major* lipophosphoglycan (LPG) interacting with a midgut expressed galectin. In contrast, the vast majority of sand flies are permissive vectors, which can support the development of many *Leishmania* species. Recently, glycan to glycan interactions involving LPG have been shown to participate in the attachment process within permissive vectors. Here, we explore the potential of *Leishmania* to co-opt host blood components to the parasite glycocalyx as a supplementary mechanism of attachment. Specifically, this project looked at the role of human pentraxins, C-reactive protein (CRP) and Serum Amyloid P (SAP) as possible cross-linkers of *Leishmania* to the sand fly midgut. Pentraxin interactions with purified LPG from nectomonad and metacyclic stage *Leishmania mexicana* parasites were investigated using a range of binding assays (western blot, ELISA and Surface Plasmon Resonance (SPR)). SAP was found to bind nectomonad LPG in a concentration- and calcium-dependent manner, with little binding to metacyclic LPG. CRP was found to bind both nectomonad and metacyclic LPG, with a greater binding capacity for the latter. The interaction between CRP and LPG could be reduced using LT6, an antibody specific to [-6Galβ1,4Manα1-PO4-]x repeats. Initial confocal experiments using log phase promastigotes show CRP binding to

the whole surface of wild type *L. mexicana*, whereas SAP only binds to a subset of parasites. Purification of *Lutzomyia longipalpis* midgut microvillar proteins and detection with SAP has revealed two possible binding targets. Furthermore, SPR analysis of midgut microvillar proteins reveal that SAP, but not CRP, can bind to *Lu. longipalpis* midguts in a dose-dependent manner. The ability of both SAP and CRP to bind nectomonad LPG indicates they both could be parasite-midgut cross-linking candidates. However, as CRP seems to bind most promastigote stages as well as the transmissive metacyclic stage, it is unlikely to be involved in midgut attachment. SAP seems to have more stage-specific binding, but further experiments are needed. Early evidence suggests SAP can also bind to *Lu. longipalpis* midgut proteins, increasing confidence in its potential role as a cross-linker. If a cross-linking agent can be identified for *Leishmania* to permissive sand fly midguts then interventions can be tested to prevent attachment and stop the *Leishmania* lifecycle, preventing further transmission.

Keyword PENTRAXINS; SAP; MIDGUT; LIPOPHOSPHOGLYCAN; TRANSMISSION-BLOCKING.

P4-051: METABOLOMIC PROFILING OF ACUTE, INDETERMINATE AND CHRONIC PHASES OF INFECTION BY *Trypanosoma cruzi* IN BALB/C MOUSE MODEL

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This work describes the clinical, parasitological and histological characterization of the infection phases in the Balb/c mice model. In addition, to understand the biological processes that lead the infection phases, we identified the metabolite profile in serum samples of infected and non-infected Balb/c females from acute, indeterminate and chronic phases of infection analyzed by HPLC-ESI-MS/MS. Seventeen metabolites were expressed while in the indeterminate and chronic phases were expressed 135 metabolites and 61 metabolites, respectively. These metabolites correspond to metabolic pathways for tryptophan, purines, glycolysis, fatty acids, arachidonic acid, arginine, glutathione, serine, sphingolipids, calcium, bile acids biosynthesis and digestive metabolism, namely: glycyl leucine, gamma amino butyric acid, methoxy-indole acetate and pyruvate indole (reported here for first time in the infection model). Results suggest that the Balb/c infection model can be used to evaluate drug candidates that are effective in the acute and indeterminate phases of infection, i.e. before organ and tissue damage occurs. On the other hand, the identified metabolites can be used as biomarkers not only for diagnosis but also for treatment follow-up and prognosis of the disease.

Keywords UNTARGET METABOLOMICS; PROFILE BIOMARKERS; CHAGAS DISEASE

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P4-052: REVEALING NEW PLAYERS IN ANTIMONY, MILTEFOSINE, AND AMPHOTERICIN B RESISTANCE IN *Leishmania* USING THERMAL PROTEOME PROFILING

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In the absence of an effective vaccine, the control of leishmaniasis is exclusively reliant on chemotherapy. In the absence of reliable molecular or genetic markers related to parasite resistance, clinical treatment failure is often taken as an indicator of drug resistance causing serious public health problems in endemic areas. Antimonial drugs have been the standard treatment for seven decades leading to major drug-resistance consequences in the Indian subcontinent. Likewise, drug resistance to miltefosine and amphotericin B continue to alarmingly spread in several continents. In consequence, innovative approaches are needed to accelerate the identification of antimicrobial drug targets and resistance mechanisms. To this end, we have implemented a novel, unbiased approach based on thermal proteome profiling (TPP) to further characterize the mode of action of antimony, miltefosine, and amphotericin B, as well as to better understand the mechanisms of drug resistance deployed by *Leishmania* to survive to these drugs. This approach combines the principle of the cellular thermal shift assay with quantitative mass spectrometry to monitor drug-target interactions using whole-parasite protein extracts. TPP is based on the principle that proteins become more resistant to heat-induced

denaturation when complexed with a ligand. In this way, we have used multiplexed quantitative mass spectrometry-based proteomics to monitor the melting profile of thousands of expressed soluble proteins in WT, antimony-resistant, miltefosine-resistant, and amphotericin B-resistant *L. infantum* parasites, in the presence (or absence) of the above-mentioned drugs. Leishmanial proteins were recovered by repeated freeze-thawing cycles and mechanical shearing. Each sample was divided in two subsamples: drug-exposed proteins and control. Samples were then subjected to a heat gradient treatment (7 different temperatures ranging from 37 to 70 °C) to induce protein aggregation, and the soluble protein fraction was recovered by ultracentrifugation. Proteins were reduced, alkylated, and digested with trypsin, and the resulting peptides were labeled using a dimethyl labeling strategy. TPP analyses were performed using a hybrid Quadrupole-Orbitrap mass spectrometer. Bioinformatics analyses were performed, including data normalization, melting profile fitting, and identification of proteins that have changed (fold change > 5) caused by complexation with a drug. Of note, thanks to this unique approach, we have been able to narrow down the regions of the proteome that interact with antimony (19 proteins), miltefosine (30 proteins), and amphotericin B (8 proteins); validating previously identified drug targets and unveiling novel ones. Moreover, these analyses revealed several candidate proteins potentially involved in drug resistance (5 for antimony, 7 for miltefosine, and 8 for amphotericin B). Interestingly, we detected thermal proximity coaggregation for several proteins belonging to the same metabolic pathway (i.e., Tryparedoxin peroxidase and Aspartate amine transferase in proteins exposed to antimony), highlighting the importance of these pathways, and providing additional information about protein interactions when parasites encounter drug-induced stress-induced. Collectively, our results could serve as a jumping-off point for the future development of novel diagnostic tools for the detection and appraisal of antimicrobial-resistant *Leishmania* populations, as well as to open the door to new on-target therapies.

Keywords LEISHMANIASIS; THERMAL PROTEOME PROFILING; DRUG RESISTANCE; PROTEOMICS; TREATMENT

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P4-053: ANALYZING COMPENSATORY TRANSCRIPTOMIC AND TRANSLATIONAL RESPONSES IN BRAZILIAN *Leishmania infantum* ISOLATES SHOWING A SPONTANEOUS 12KB-DELETION

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Visceral leishmaniasis in the American continent is a severe disease, caused by the unicellular protozoan parasite *Leishmania infantum*. These parasites were introduced to the America Continent around 500 years ago, during the European conquest of the continent. We are applying a comparative genomics approach to investigate how *L. infantum* evolved in this new environment, and how ecological adaptation influences today's infection dynamics and clinical disease outcome. Sequencing analysis of well-adapted and highly dispersed *L. infantum* strains from Brazil revealed a spontaneous deletion on tetrasomic chromosome 31, causing the loss of four open reading frames (ORFs), i.e. the ecto-3'-nucleotidase (LINF_310031200), ecto-3'-nucleotidase precursor (LINF_310031300), helicase-like protein (LINF_310031400), and 3-2-trans-enoyl-CoA isomerase (LINF_310031500) genes. Here we assess the consequences of this deletion on parasite phenotype, transcript profile, and protein translation by applying gene editing and comparative systems analyses on natural deleted (DEL) and non-deleted (Non-DEL) Brazilian *L. infantum* isolates as well as Old World strains from Tunisia (Non-DEL). We will evaluate the phenotype of DEL strains following reconstitution of the four ORFs, and re-engineer the DEL genotype in wildtype *L. infantum,* aiming to determine the role of the ORFs in parasite viability, infectivity, and fitness. Moreover, we will combine DNA-

and RNA-seq analyses with ribosome profiling to explore possible mechanisms that compensate for the genomic deletion. Results on the effect of deleting the four open reading frames on parasite fitness, as well as transcript profiles from DEL and Non-DEL strains will be shown. Our research will provide new insight into how gene deletion and possible compensatory responses drive parasite fitness gain in general and shaped *L. infantum* ecological adaptation and disease dynamics following its introduction into the New World.

Keywords *Leishmania infantum*; DELETION; ADAPTATION; GENOMICS; TRANSCRIPTOMICS

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P4-055: HIGH COMPLEXITY BARCODING AND SINGLE-CELL GENOMICS REVEAL THE CLONAL DYNAMICS OF *Leishmania donovani* ADAPTATION TO ANTIMONY PRESSURE *IN VITRO*

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Drug resistance (DR) represents a major threat for clinical management and control of leishmaniasis. Worryingly, *in vitro* experimental studies have demonstrated the parasite's ability to quickly develop resistance to all drugs now available, including combinations of them and new experimental compounds. Drivers of DR have been identified, but information on the population dynamics of emergence and spreading of DR is lacking. We hypothesized that the ability of *Leishmania* to quickly generate cellular heterogeneity – in particular through changes in chromosome copy number – plays a role. To investigate this, we performed a 'flash selection' (FS) of antimony resistance in *L. donovani*: this protocol consists of continuously exposing parasites to 382 µM of trivalent antimony for 5 consecutive passages. When applied to naturally tolerant strains (like those identified in the Indian subcontinent harboring natural amplification of the MRPA locus), FS allows to select rapidly lines highly resistant to antimony. To study population dynamics during FS, we developed and applied a high complexity cellular barcoding method: individual cells are tagged with unique DNA sequences, allowing to track the evolution of more than 500 intra-clonal lineages. This barcoded population was divided into 4 independent replicates that underwent FS and were followed (together with un-exposed controls) during the 5 passages. FS led to a drastic reduction in lineage

diversity, with 337 lineages being negatively affected by the drug consistently among the 4 replicates, indicating pre-existing lower antimony tolerance in these specific lineages. In contrast, 39 lineages not only survived to FS, but also displayed a fitness gain under antimony pressure. Most of these lineages were different in each replicate, suggesting that a fraction of the parasite population -with cells marked by different barcodes- showed a similar and higher drug tolerance, with their further expansion being stochastically driven. Bulk whole genome sequencing revealed that drastic changes in aneuploidy, which quickly emerged in all 4 replicates, were the only consistent genomic alteration associated with antimony pressure. Moreover, these changes in bulk aneuploidy coincided with the changes in the dominant lineages in each population. Although different replicates displayed different bulk aneuploidy profiles, all replicates shared an increase in the copy number of chromosomes 23, 27, and 31, indicating a potential selective advantage of the amplification of these chromosomes. Noteworthy, chromosome 23 bears an amplicon containing the MRPA gene, a well-known driver of antimony resistance; dosage increase of chromosome 23 thus results in a further amplification of this key locus. To investigate whether the measured aneuploidy changes were pre-existing or generated de novo, we inspected the results of high-throughput single-cell genome sequencing of the population prior to FS. We did not find the emerged karyotypes in the starting population, suggesting that corresponding changes in aneuploidy are generated de novo. Together, our data suggest that adaptation to antimony *in vitro* is initiated by the selection of a specific and more tolerant sub-population of parasites. Further adaptation is driven by the stochastic evolution of lineages, potentially strengthened by the aneuploidy changes emerging de novo in these lineages.

Keywords DRUG RESISTANCE; CLONAL DYNAMICS; LINEAGE TRACKING; SINGLE-CELL GENOMICS; ANEUPLOIDY

P4-056: COMBINING MULTI-LEVEL BIOINFORMATICS TOOLS TO GLOBALLY ASSESS THE DIVERSIFIED CRITICAL ROLES OF SIDER2 RETROPOSON ELEMENTS IN *Leishmania* GENOME PLOIDY, GENE EXPRESSION AND DEVELOPMENTAL REGULATION

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We have previously reported that the *Leishmania* genome harbors a large number of truncated versions of formerly active retroposons termed SIDERs (Short Interspersed DEgenerated Retroposons). SIDERs are divided into two distinct subfamilies, SIDER1 and SIDER2 fulfilling different functions in the cell. SIDER2 elements were shown to regulate mRNA turnover. To allow a better genome-wide characterization of the SIDER2 subfamily, we used a multi-level bioinformatics approach along with comparative genomic, transcriptomic and proteomic profiling of *L. infantum* promastigotes, axenic amastigotes and macrophage-derived amastigotes. Our bioinformatics pipeline consisted of using Hidden Markov Model to identify SIDER2 homologs and fragments across a recent version of the *L. infantum* genome (TriTrypDB-56_LinfantumJPCM5). The results provided a more accurate and detailed map uncovering 1449 SIDER2 elements regularly dispersed along the chromosomes in a generally synthetic distribution. 1128 (77.8%) were located within 3'UTRs of which 948 (85%) in sense orientation and 180 (15%) in the antisense orientation. Prediction of polyadenylation and *trans*-splicing sites was performed using the *PRED-A-TERM* algorithm. Illumina RNA-seq analysis confirmed 1127 (99.99%) of the predicted SIDER2-bearing mRNAs and their 3'UTR location. RNA-seq from *L. infantum* promastigotes and amastigotes allowed the first genome-wide comparative expression analysis of SIDER2- and non-SIDER2-containing transcripts. Remarkably, SIDER2-mRNAs were generally less

expressed than non-SIDER2 mRNAs ($p < 0.001$) in promastigotes but also in amastigotes, confirming the major role of SIDER2 in mRNA degradation. Comparable expression levels were observed between mRNAs harboring sense and antisense SIDER2 suggesting structural requirements for SIDER2-mediated mRNA decay. Preliminary sequence-structural clustering revealed possible subsets of SIDER2 elements sharing particular secondary structure with related expression levels. Comparative analysis between promastigote and axenic amastigote transcriptomes revealed 146 up regulated SIDER2-containing transcripts and 98 were up regulated when comparing promastigotes to intracellular amastigotes. Among the up regulated genes, 60 were common to axenic and intracellular amastigotes. Interestingly, SIDER2-containing transcripts up regulated only in axenic amastigotes (exposed to temperature and acidic pH stress) disclosed different predicted functions from those expressed solely in the adapted macrophage-derived amastigotes. While organelle envelope related functions were associated with genes upregulated in axenic amastigotes, an enrichment for transporter activity was observed in intracellular amastigotes. There was also functional clustering when comparing low-expressed to high-expressed (inactive SIDER2) SIDER2 transcripts. Furthermore, a motif search analysis revealed different motif organization between low and high expressed SIDER2 transcripts. Comparative data analysis of label-free quantitative proteomics and RNA-seq showed a good correlation between SIDER2-containing mRNA expression and protein abundance, indicating that SIDER2 transcripts are mostly regulated at the RNA level. To assess the impact of SIDER2 elements on chromosome copy number variation (CNV), we compared *L. infantum* Illumina DNA-seq to the RNA-seq data. This analysis supports that the presence of low-expressed SIDER2 transcripts could force CNV as a compensatory mechanism. This was the case for Chr 31 (4 copies) that exhibited the highest percent of low expressed SIDER2-mRNAs. Altogether, these data provided a detailed characterization of SIDER2 elements in *L. infantum* and demonstrated their central role in regulating genome ploidy and gene expression at the post-transcriptional level.

P4-057: IDENTIFICATION OF VIRAL SEQUENCES FROM METATRANSCRIPTOMIC DATA OF THE INSECT VECTOR *Lutzomyia longipalpis*

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Lutzomyia longipalpis is an insect vector with a relevant role in the transmission of visceral leishmaniasis in America. Considering that it is a species of epidemiological interest and that arboviruses have been previously reported within the sandfly group, there is little information on the set of viruses that can be found within this species in Colombia. In this study, a metatranscriptomic analysis was performed to identify viral sequences present in samples of *Lu. longipalpis* from Ricaurte, Cundinamarca, Colombia. Groups of 10 female individuals were formed for analysis and subsequent RNA extraction and sequencing. An average of ~34 million sequenced reads per sample were obtained, which were processed by the SortMeRNA and BWA programs to filter the sequences associated with the host and ribosomal RNA. Finally, the remaining reads were assembled using the MetaviralSPAdes program for the identification of viral sequences. Two strategies based on alignments and search for sequence signatures were used to classify virus-associated sequences. Here, we identified two sequences related to the families of *Mononegavirales* and *Hepelivirales* from an analysis of the RdRp enzyme. The RNA-seq technique

allowed, in addition to the identification of viral sequences, the taxonomic confirmation of the host using the COI analysis and the detection of natural infection by *Leishmania* (kDNA) through the directed search of sequences in females of *Lu. longipalpis*. The results show that the RNA-seq technique is a powerful tool that can detect multiple parasites and generate host organism information at the genomic and transcriptomic levels. Two viral sequences were found, which increases the knowledge of the set of viruses present in this insect vector. It would be desirable to carry out an additional molecular analysis of the viral sequences found, which have not been registered in Colombia, to characterize them, verify the active infection within the insect cells, and describe its effect on the organism and its associated microorganisms.

Keywords *Mononegavirales*; *Hepelivirales*; *Lutzomyia longipalpis*; RNA-SEQ

P4-058: WHOLE BLOOD TRANSCRIPTOMIC PROFILE OF NON-ULCERATED CUTANEOUS LEISHMANIASIS CAUSED BY *Leishmania (L.) infantum chagasi* IN AMAPALA, HONDURAS

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Non-ulcerated cutaneous leishmaniasis (NUCL) is a rare form of leishmaniasis described in areas of visceral leishmaniasis (VL) transmission in Central America, including Honduras, Costa Rica, El Salvador, and Nicaragua. *Leishmania infantum* is implicated as the etiological agent of both clinical outcomes. Parasites are transmitted by *Lutzomyia longipalpis* sand flies that bite the vertebrate hosts. The outcome of the infection could be related to the host immunogenetic backgrounds, as well as parasite and sand fly vector features. An approach to the parasite-host interaction at the gene expression level could improve the understanding of the host response definition. Therefore, the aim of this study was to compare the transcriptome in whole blood from human patients with NUCL and VL by RNA-seq from Illumina. For that, 10 NUCL, two VL, 9 asymptomatic patients from Amapala municipality, Honduras and 7 non-infected patients from São Paulo, Brazil were investigated by serological (ELISA) and molecular parasitological (PCR) diagnosis. The preliminary unsupervised principal component analysis of the gene expression level revealed 3 clusters: an

exclusive cluster of VL cases, a cluster including NUCL and asymptomatic and an exclusive cluster of non-infected. The number of disturbed genes after *L. infantum* infection was significantly higher in VL than in NUCL and asymptomatic. The comparison of gene expression levels between LCNU and VL generated a list of 2-fold change differently expressed genes (DEGs), which was grouped in the functional enrichment analysis using the Reactome L3, Reactome Immune System and BTM databases. The enriched pathways were grouped into sets of 8 pathway clusters significantly upregulated [normalized enrichment score (NES > 0, $P_{adjusted} < 0.05$) and 14 pathway clusters of significantly downregulated (NES < 0, $P_{adjusted} < 0.05$), based on function. In order to understand the role of significant pathways for the clinical outcome, the NES values were compared between clinical groups in NUCL x Neg and VL x Neg contrasts. Highlighted, the pathway involved in neutrophil degranulation was more downregulated in NUCL and the pathways involved with IL-10 and TGF-b signaling were more downregulated in VL. In contrast, pathways related to $CD4^+$ T lymphocyte activation and proliferation, activation of B lymphocyte, plasma cells and immunoglobulin production, activation and triggering of the classical pathway of the complement, myeloid cells activation by FCER1 and FCGR, non-canonical NFk-b signaling mediated by dectin – 1, cross-presentation of exogenous soluble antigen were more upregulated in VL than in NUCL. Pathways involved with MHCII - antigen presentation, JACK-STAT signaling after stimulus with IL-12 and scavenging of heme from plasma were also downregulated in NUCL x VL with no indication of upregulation or downregulation in NUCL x Neg or VL x Neg contrasts. In summary, these data corroborate the findings of our group on immunopathology and point to a transcriptomic profile in the whole blood related to the effective control of the immune response and prevention of tissue damage in NUCL, while occurs a less controlled multi-systemic immune activation state in VL.

Keywords NON-ULCERATED CUTANEOUS LEISHMANIASIS; VISCERAL LEISHMANIASIS; TRANSCRIPTOME; *Leishmania (L.) infantum chagasi*; RNA-SEQ

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P4-060: TRANSCRIPTIONAL APPROACH UPON TLR2, CHEMOKINES AND NEUTROPHILS IN HUMAN VISCERAL LEISHMANIASIS

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Toll-like receptors (TLRs) are a family of pattern recognition receptors (PRRs) that are mainly expressed in presenting cells and phagocytes, such as dendritic cells, macrophages and neutrophils. TLRs are involved in the generation and regulation of innate immunity during *Leishmania* infection and influence the subsequent pathogen-specific immune response that play a pivotal role in the outcome of infection. Neutrophils express numerous PRRs, including TLRs which, after engagement with pathogen-associated molecular patterns (PAMPs) on the surface of the *Leishmania* parasite, lead to the activation of the microbicidal responses of neutrophils. The goal of this study was to evaluate the expression of TLRs and the enriched pathways relative to neutrophils in human infection caused by *Leishmania (L.) infantum chagasi*, analyzing the blood transcriptome (RNA-seq) of individuals from an endemic Brazilian Amazon region (Bujaru municipality, Pará state) for visceral leishmaniasis (VL), including active VL patients (VL, n=10), and infected asymptomatic individuals (AI, n= 15) with high response to the intradermal leishmanin test and low production (less than the cut-off titer 80) of specific antibodies. The mRNA expression of both

groups was compared to that found in healthy endemic controls (n=11). In addition, we determined the parasite load in both clinical groups by q-PCR. Here, we detected a significant downregulation (Padjusted < 0.05) of TLR2 (Log2FC= - 0.60), CXCL1 (Log2FC= - 0.85), IL-8 (CXCL8) (Log2FC= - 1.89), CXCR1 (Log2FC= - 1.60), and CXCR2 (Log2FC= - 2.01) in the active VL patients, contrary to AI individuals, who showed significant upregulation (Padjusted < 0.05) of all these genes (TLR2 Log2FC= 1.44), (CXCL1 Log2FC= 0.82) (IL-8 (CXCL8) Log2FC= 0.84), (CXCR1 Log2FC= 1.20), (CXCR2 Log2FC= 0.95). Besides, the proportion of neutrophils (deconvolution tool) were higher in AI compared to VL patients (P< 0.05), and the parasite burden was at least 50-fold lower in AI than in VL group. Neutrophils are the first cells recruited to *Leishmania* inoculation site and effectively participate in controlling the parasite growth and survival. Chemokines CXCL1 and Interleukin-8 (CXCL8) are powerful neutrophils chemotactic factors, and CXCR1 and CXCR2 are the major chemokine receptors on neutrophils. Moreover, previous studies also demonstrated that TLR2 has a direct effect on neutrophils, mediating their activation and production of leishmanicidal molecules. Here, all these genes were overexpressed in the asymptomatic infection, the most prevalent profile found in endemic areas, and downregulated in active VL. Based on the present data set, we suggest that TLR2, chemokines CXCL1 and IL8 (CXCL8), and their respective receptors CXCR1 and CXCR2 act cooperatively to recruit and activate neutrophils, inducing host protection in human *Leishmania (L.) infantum chagasi* infection.

Keywords HUMAN VISCERAL LEISHMANIASIS, TOLL-LIKE RECEPTOR 2, CHEMOKINES, NEUTROPHILS, BLOOD TRANSCRIPTOMICS

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P4-061: TRANSCRIPTOMIC ANALYSIS OF CUTANEOUS LEISHMANIASIS LESIONS IDENTIFIES PATHWAYS ASSOCIATED WITH THERAPEUTIC OUTCOME

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The immune response is central to the pathogenesis of cutaneous leishmaniasis (CL). However, most of our current understanding of the immune response in human CL derives from analysis of systemic responses, which only partially reflect what occurs at the skin. Here we sought to characterize the transcriptional dynamics of skin lesions during the course of treatment of CL patients, and to identify gene signatures and pathways associated with healing and non-healing responses. We performed a comparative transcriptomic profiling of serial skin lesion biopsies from CL patients obtained before, at the middle and at the end of treatment. Of 12 CL patients evaluated, 5 cured and 7 presented with therapeutic failure. A principal component analysis (PCA) showed a significant amount of the variance between visits within the cured group, while no marked discrimination between visits was evident in the failure group. In cured patients, the number of differentially expressed (DE) genes was the highest between pre-treatment biopsies (B1) compared to middle of treatment (B2) and end of treatment (B3) (674 and 1170 DE genes, respectively). On the contrary, minimal transcriptional changes (23 DE genes) were observed between biopsy samples obtained at middle of treatment (B2) vs. end of treatment (B3). In patients with treatment failure, the most significant transcriptional changes occurred between B1 and B3 (1630 DE genes), while few genes were DE at the middle of treatment (B2 vs. B1, 146 DE genes

and B2 vs. B3, 89 DE genes). Enrichment analysis of DE genes throughout treatment (B2vsB1 and B3vsB1) in lesions from patients who healed revealed upregulation of formation of the cornified envelope, collagen biosynthesis and formation of the extracellular matrix, retinoic acid metabolism, ceramide synthesis, and IL1F signaling. Downregulated genes were predominantly related to IL10 signaling, neutrophil degranulation, metallothioneins, and T cell co-stimulation. These data suggest that antileishmanial-mediated healing of dermal lesions is dependent upon development of the stratum corneum, suppression of Tcell-mediated inflammatory response and dampening of neutrophil activation. In patients with treatment failure, genes involved in transcriptional regulation of white adipocyte differentiation and glutathione conjugation were induced, while genes associated with cell cycle progression, interferon signaling and cellular senescence were downregulated. This work provides insights into the factors that contribute to the effective resolution of skin lesions caused by *L. Viannia* and the identification of interventional therapeutic targets.

Keywords *Leishmania Viannia*; SKIN BIOPSIES; RNA-SEQ; TRANSCRIPTIONAL DYNAMICS

P4-062: THE CLINICAL-IMMUNOLOGICAL SPECTRUM OF HUMAN *Leishmania (L.) infantum chagasi* INFECTION IN THE BRAZILIAN AMAZON: A TRANSCRIPTOMIC APPROACH

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In the Brazilian Amazon, the clinical-immunological spectrum of human *Leishmania (L.) infantum chagasi* infection has been defined by our group on species-specific and semi-quantitative DTH/IFAT-IgG assays and clinical evaluation of infected individuals. The spectrum comprises five profiles: three asymptomatic, intitled as Asymptomatic Infection (AI=$DTH^{+/++++}/IFAT^{-}$), Subclinical Resistant Infection (SRI= $DTH^{+/++++}/IFAT^{+/++}$), and Indeterminate Initial Infection (III= $DTH^{-}/IFAT^{+/++}$); and two symptomatic, named Subclinical Oligosymptomatic Infection (SOI) and Symptomatic Infection (SI=American visceral leishmaniasis - AVL), both with the same immunological responses ($DTH^{-}/IFAT^{+++/++++}$), but distinct clinical presentation. Aiming to improve our knowledge on this spectrum and also verify whether our approach in endemic areas would be supported by distinct transcriptional patterns respective to the five evolutive profiles of infection above described, we analyzed the whole blood gene expression of 56 individuals from the

endemic area of Bujaru municipality, northeast of Pará State (Brazil), comprising 19 III, 9 SRI, 15 AI, 3 SOI and 10 SI (=AVL). In addition, 11 blood samples of healthy individuals from the same endemic area were collected for comparisons (control group). For that, next-generation RNA sequencing (RNAseq) on the Illumina platform was performed, followed by bioinformatics analysis. Blood transcriptomics showed a large number of differentially expressed genes (DEGs) for each group: 1,148 up-regulated and 324 down-regulated DEGs in AI profile, 1,350 up-regulated and 171 down-regulated DEGs in SRI, and 1,849 up-regulated, 234 down-regulated DEGs in SOI profile. The highest amount of DEGs was detected in SI profile (=AVL) with 1,341 up-regulated and 1,210 down-regulated genes, and the lowest one in III profile, which presented 243 up-regulated and 407 down-regulated genes. Heatmap including all DEGs showed five different patterns of gene expression, corresponding to each clinical-immunological profile. Moreover, Venn diagram revealed groups of DEGS exclusively up or down-regulated in each profile (Total AI= 874; SRI=132; III= SOI=700; AVL=1,950) that were related to biological processes, including specific genes of the innate and acquired immune response, such as those related to Toll-like receptors activation, reactive oxygen species production, chemokines and cytokines expression, among others. In summary, the blood transcriptome analysis of human *L. (L.) infantum chagasi* infection showed distinct gene expression patterns and exclusive DEGs for each clinical-immunological profile that associated with the outcome of infection. Finally, our present transcriptomic findings sustain our clinical-immunological approach that has been accomplished in endemic areas for recognizing the five evolutive profiles of the human infection caused by the parasite, which includes three asymptomatic and two symptomatic stages

Keywords *Leishmania (L.) infantum chagasi*; HUMAN INFECTION; CLINICAL-IMMUNOLOGICAL PROFILES; GENE EXPRESSION

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P4-063: TRANSCRIPTOME-BASED IDENTIFICATION OF SNPS BIOMARKERS FOR PHENOTYPIC CLUSTERING OF *Leishmania* (*Viannia*) *panamensis*

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The search for genetic markers of drug resistance in *Leishmania* has been long pursued. However, no clear association between drug susceptibility phenotype and the therapeutic response and has emerged and impede the use of drug susceptibility in treatment policy or decisions for control and management of leishmaniasis. We conducted multiplexed transcriptome sequencing of >60 clinical strains of *L. V. panamensis*, isolated from patients with known outcomes of treatment with meglumine antimoniate, with phenotypic profiles of isoenzyme electrophoretic mobility (zymodems) and whose drug susceptibility phenotype was characterized. Candidate gene selection for population clustering was based on the number of SNP variants/gene and their ability to discriminate between two zymodeme subpopulations (2.2 and 2.3). *Leishmania* SNP genotypes associated with subpopulations were used to design a PCR-based SNP screening tool. Analysis of transcriptomes from 65 *L. V. panamensis* clinical strains revealed clustering according to the zymodeme phenotypic profile, and this was observed when analyzing differentially expressed (DE) genes or SNP profiles. DE genes were predominantly found within four categories: metabolism, nutrient transport, redox balance and immunogenicity. Of DE genes, 10 were selected to design sequencing primers for PCR-based SNP screening. Among these, one marker was discriminatory based on

presence/absence of the amplification product, while the other nine were dependent on SNP genotyping.

Keywords *Leishmania Viannia*; RNA-Seq; ZYMODEMS; CLINICAL STRAINS

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5.7. SOCIAL INNOVATION - IMPLEMENTATION RESEARCH - OPERATIVE RESEARCH

P2-099: THE GLOBAL HEALTH AGENDA AND THE LEISHMANIASIS CONTROL PROGRAM IN COLOMBIA

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Leishmaniasis is considered by the World Health Organization (WHO) as an important infectious disease and a global health problem. In 2015, the United Nations (UN) established a commitment, through the Sustainable Development Goals (SDGs), to ensure healthy lives and promote well-being for all by 2030. One of the goals is to end epidemics of neglected diseases, such as leishmaniasis, but control programs have not been effective in containing their geographic expansion and growing urbanization. In Colombia they have become endemic in almost all of its territory. Estimates point to around 11 million people at risk of contracting the disease, mainly in rural areas where three clinical types predominate, whereas the most frequent and with the widest geographical distribution is cutaneous leishmaniasis (95% to 98% of cases), followed by the form mucocutaneous (1% to 4%) and the visceral form (0.1% to 1.5% of cases). This exploratory and descriptive study aims to contextualize the actions of control and confrontation of leishmaniasis by institutions, government agencies and other social actors. The methodology uses a critical and interdisciplinary approach via documental research and interviews in order to identify historical-social processes to analyze the most relevant theoretical, experimental and practical works produced by the main research institutions in Colombia. An analysis was made of the control actions carried

out by scientific and health institutions by the end of the 20th century and the production of knowledge about the spatial distribution of leishmaniasis, its vectors, parasite species and hosts and the way in which these studies subsidized the elaboration of the Leishmaniasis Control Program in Colombia. Relevant aspects of the research are: epidemiological surveillance - the detection of cases until the analysis of data and indicators to characterize the distribution of the disease and its clinical and epidemiological profile; interruption in the transmission chain; administrative aspects; the development of vaccines and preventive measures, such as the use of repellents, mosquito nets, the elimination of vectors, wild and domestic hosts and the application of insecticides. The disease mainly affects the population in vulnerable situations. Treatment is prolonged and painful. The therapy uses pentavalent antimonials (Sb5+), such as Nmethyl antimoniate glucamine and sodium stibogluconate, effective but toxic drugs that can cause serious side effects. Pentamidine stibugluconate and amphotericin B are used when there is resistance to conventional treatment. There are other drugs whose results have been promising in clinical trials, such as miltefosine, administered orally, which had cure rates above 80%, and paramomycin, for topical use, with cure rates between 74% and 85%. In isolation, national states such as Colombia have difficulties in effectively dealing with the expansion of leishmaniasis, and prevention measures have proved to be limited and insufficient. However, the commitments made to control these diseases, in close connection with the achievement of the SDG targets, have the potential to improve the living and health conditions of populations through the reduction of inequalities.

Keywords HISTORY OF LEISHMANIASIS; GLOBAL HEALTH; LEISHMANIOSIS CONTROL PROGRAMS; SUSTAINABLE DEVELOPMENT GOALS; HISTORY OF COLOMBIA

P2-100: GEOSPATIAL ANALYSIS BY GIS. IN THE BEHAVIOR OF CUTANEOUS LEISHMANIASIS, IN RURAL COMMUNITIES, BENCHMARK FOR DESIGNING STRATEGIES FOR HEALTH EDUCATION

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Cutaneous Leishmaniasis represents a public health issue in Latin America, especially in rural areas, Communication and information technology strategies (CITs) in health care education are essential for prevention and control of communicable diseases. From this approach, a field research in education and community participation in cutaneous leishmaniasis was carried out in La Hoyadita, a rural area of the Hatillo Municipality, Miranda state Venezuela. The methodology applied is an epidemiological ethnographic type, supported by a Participatory Geographic Information System (P-GIS), as a facilitating the analysis of the strategic geospatial worldview by the population, the basis for developing sustained education and community participation designs focused on cutaneous leishmaniasis Due to the nature of the research, we worked from the perspective of a transdisciplinary team, and with the principles of triangulation. Triangulation of: A) Approaches to Theories. B) Methods. C) Researcher Approaches. D) Tools/Techniques: Operationalization of the PGIS, such as: GPS. Official cartography of the area, and 1:100,000 scale orthophotomaps. Basic maps prepared by the local health team (sketch), plotter, compass. GPS. Direct participant observation. General Community characterization forms. Open interviews with key community and institutional people. Photographic and audiovisual records. Community Assemblies. Analysis of local and municipal health data. E) Analysis of the Results/data: Analysis of trends of comprehensive scenarios, articulating the clinical-epidemiological

indicators of health and particularly in leishmaniasis, the ethnographic analysis of the worldview of geospatiality and health, by the inhabitants in a stratified way by sectors supported by thematic maps and an audiovisual database. Among the results obtained, it can be evidenced that the application of GSI, as a resource for geospatial analysis, facilitated the identification of a significant disagreement in the worldview of geospatiality, manifested by the different key social actors in this study: the team of local health, the villagers; reflected not only in the boundaries of the communities, but also in their sociodemographic relationship and the identification of active and suspected cases of leishmaniasis. This study reflects the geostrategic value of the worldview of space as a unit of analysis in health, from local institutions such as the Ambulatory, the School and the communities. If we manage to stratify the communities socio-demographically, we have better scenarios to design a leishmaniasis control program in a sustainable way, using timely information reflected in maps which have been prepared jointly by researchers and communities.

Keywords GEOSPATIALITY; EDUCATION; COMMUNITY PARTICIPATION; LEISHMANIASIS

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P2-101: COST OF ILLNESS OF VISCERAL LEISHMANIASIS TO PATIENTS AND THEIR FAMILIES IN NORTHWEST ETHIOPIA

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Visceral Leishmaniasis (VL) is an important public health problem in Ethiopia, mainly affecting daily laborers and farmers residing in VL Leishmaniasis endemic areas. Although VL drugs are free of charge in Ethiopia, patients and their families incur costs during health care seeking and from income-loss due to illness. The aim of this study was assess the direct and indirect cost of illness of VL patients and their families in Northwest Ethiopia. We conducted an institution based cross-sectional study including adult VL patients from the three main VL treatment centers in Northwest Ethiopia (University of Gondar Hospital, Abdurafi Health Center and Metema Hospital) between September and December 2015. Data were collected by trained study staff and included direct (medical and non-medical) and indirect costs collected separately for the period before and after VL diagnosis. Of the 403 VL patients included in the study, 391 (99.2%%) were male; the median age was 25 years. Of these, 228 (59 %) were daily labourers and 128 (33%) farmers. Their median household income was 56 USD (IQR 30-110). The medium time to diagnosis was 62 days (IQR 40-60); the median number of health care providers visited before VL diagnosis was 2 (IQR 1-4). The first type of health care provider/service visited mostly consisted of a public primary health center (n=222; 56.7%), a private clinic (n=109; 27.9%) or a traditional healer (n=60; 15.4%). The total direct cost combined was 53 USD (IQR 21-104). The median total direct

cost to patients before VL diagnosis was 40 USD (IQR 16-70), consisting of 31 USD (IQR 13-52) of medical direct cost (consultation, laboratory investigation and medication) and 9 USD (IQR 3-17) of non-medical medical (transport and food). For VL diagnosis and treatment, the median direct cost was 13 USD (IQR 4-34), consisting of 4 USD (IQR 2-9) of medical direct cost and 9 USD (IQR 3-17) of indirect medical costs. Patients could not carry out normal daily activities for a median of 50 days (IQR 30– 80), with a median loss of income of 98 USD IQR 58-150). Caretakers reported a median loss of 27 workdays (IQR 10–35), with a median loss of income of 57 USD (IQR = 16.7-40). In total, the median income lost (indirect costs) for the family per episode of VL was 128 USD (IQR 82-208). The overall cost related to the VL episode was 181 USD (IQR 103-313). A total of 225 (57.5%) of households incurred catastrophic expenditure (spending >40% of the monthly income) related to the VL episode. Although VL medication is given for free, the cost of the VL episode was generally high in relation to the household income. Measures to reduce the financial impact of VL include awareness raising amongst health care providers and patients to shorten the diagnostic process, and ambulatory or shorter VL treatment regimens to reduce the duration of hospitalization and productive time lost.

Keywords VISCERAL LEISHMANIASIS; DIAGNOSIS; TREATMENT; COST; INCOME-LOSS; ETHIOPIA

P2-102: PROVIDING BETTER UNDERSTANDING OF CLIMATE AND ENVIRONMENTAL DRIVERS OF SAND FLY BORNE DISEASES – THE CLIMOS PROJECT

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Over the last two decades, four successive research consortia (EDEN, EDENext, VBORNET, and VectorNet) aimed at improving knowledge, surveillance, and control of vector-borne diseases in Europe and neighbouring countries. Among these, phlebotomine sand fly-borne diseases including leishmaniasis and phleboviruses represent an important public health and veterinary concern. CLIMOS - Climate Monitoring and Decision Support Framework for Sand Fly-borne Diseases Detection and Mitigation with Cost-benefit and Climate-policy Measures – including 30 partners across 16 countries, aims to complement and build on previous efforts, bringing together researchers, health-care and veterinary practitioners, technology platform designers and at-risk communities, to conduct innovative and applied research seeking to better prepare for current and future impacts of climate and environmental changes on human and animal health, using sand flies and the diseases they transmit as a model system. Specifically, CLIMOS aims to empirically characterise the microclimatic, demographic, and epidemiologic characteristics associated with longitudinal datasets on sand fly abundance and animal infection rates at different geographical scales across Europe and neighbouring countries. These data will feed into epidemiological-climatic predictive mathematical models of realistic human-induced climatic changes scenarios to help develop an early warning system of infection and disease designed with the input of partner public health ministries for public use. The project will develop novel technologies to monitor and mitigate human-sand fly contact.

Keywords BIG DATA; COPERNICUS; *Leishmania*; PHLEBOTOMINE SAND FLIES; *Phlebovirus*

P2-103: VULNERABILITIES TO AND THE SOCIOECONOMIC AND PSYCHOSOCIAL IMPACTS OF THE LEISHMANIASES: A REVIEW

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Leishmaniasis is a neglected tropical disease (NTD) and the second deadliest parasitic infection globally, causing approximately 650,000 DALYs annually in endemic countries. The primary presentations of leishmaniasis - cutaneous (CL) and visceral (VL), along with secondary post kala-azar dermal leishmaniasis (PKDL), are caused by parasites of the *Leishmania* genus and are spread by the bite of *Phlebotomine* sandflies. Poverty is a critical risk factor for leishmaniasis, but a comprehensive understanding of additional socioeconomic risks would provide new avenues for intervention. This study aimed to provide a contemporary narrative review of the socioeconomic drivers and impacts of leishmaniasis globally. An initial review of the literature was conducted to identify key socioeconomic themes relating to leishmaniasis followed by a deeper literature review into each theme. We identified and described five core socioeconomic themes related to leishmaniasis: economic burden, access to care, HIV coinfection, psychosocial effects, and population migration. Catastrophic healthcare expenditure (direct and indirect medical costs) compounds existing financial strain in low-income communities for both households and healthcare providers. Alleviating leishmaniasis would provide significant economic benefit from averted productivity loss on a global scale. A lack of

effective, non-invasive diagnostics to identify asymptomatic carriers allow human reservoirs to persist, perpetuating sandfly infection rate in endemic communities. Unusual clinical manifestations of Leishmaniasis in people with HIV delays diagnosis and further complicates the deleterious relationship between these two diseases. Additionally, expanded treatment options are required to address relapse rates for Leishmania-HIV coinfected individuals. The dermatological presentation of leishmaniasis may result in short or long-term severe disfigurement, leading to stigmatization and psychosocial burden. In particular, rural women often face social exclusion and discrimination, which can negatively impact social and economic opportunities tied to marriage. Climate change and political instability are major drivers of population migration, resulting in the introduction of naïve populations to endemic regions and of infected populations into non-endemic regions. Warmer temperatures provide better conditions for sandflies to reproduce and feed, allowing the vector to spread to areas previously considered safe from leishmaniasis. Five thematic socioeconomic factors both exacerbate the risk and drive the impact of leishmaniasis on endemic countries worldwide. Emerging foci demonstrate a need for continued effort to address these factors if leishmaniasis control is to be achieved. While our thematic findings were informed by literature from various countries, the majority of recent research was conducted in South East Asia and thus may not capture localized nuances in other endemic regions.

Keywords ECONOMIC-PSYCHOSOCIAL IMPACTS; KALA-AZAR; LEISHMANIASIS; NEGLECTED TROPICAL DISEASES; RISK FACTORS

P2-104: ESTIMATING RESOURCE NEEDS AND COSTS RELATED WITH MOLECULAR XENOMONITORING OF *Phlebotomus argentipes* FOR DETECTING *Leishmania donovani* IN BIHAR, INDIA

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As India prepares for post-elimination of visceral leishmaniasis (VL), molecular xenomonitoring (MX) may be a promising approach for detecting possible transmission of VL when cases continue to decline in India. MX integrates fieldwork and molecular analysis of collected vector specimens, both of which involve significant allocation of human resources, equipment, laboratory consumables and other resources. Here we describe the development of a standardized tool for assessing resource needs and costs associated with implementation of MX when using CDC light traps for collection of *Phlebotomus argentipes*, the only known sand fly vector of *Leishmania donovani* in India. We also sought to provide an estimated cost per sand fly pool analyzed. MX was divided into five main activities: trap installation, specimen collection, specimen sorting, specimen pooling, nucleic acid extraction, and molecular analysis. Each activity was further divided into sub-activities to allow for stepwise observational data collection using an Excel spreadsheet activity tracker for documentation of the human, time, and physical resources needed. Five complete observations were conducted for each of the six main activities. Data needed to determine estimates per observation included cost category (i.e. staff, equipment, consumable supplies), number per unit, currency used, duration of events, estimated lifespan of reusable supplies and equipment, and information source. Due to disruption in fieldwork related to the COVID-19 pandemic, observational time estimates were obtained using retrospective

Google Timeline data to collect distance, duration, and location level data associated with field and laboratory work that occurred during September to November 2019 and March 2020. Preparation and laboratory time estimates were also based on previous occurrences and manufacturers' protocols. Cost were obtained from invoices and proposed budgets for staff and other financial sources. Full costing analysis will be presented. The easily adaptable costing template will allow for a standardized method for accounting costs and resources for each MX activity. It will provide budget and resource allocation estimates while also monitoring project expenses to assess the cost-effectiveness of project activities. The tool may also be used in other VL endemic areas outside of Bihar, including the rest of the Indian subcontinent and endemic areas of East Africa, and may be particularly useful as vector and human surveillance efforts become more integrated.

Keywords MOLECULAR XENOMONITORING; OBSERVATIONAL COSTING ESTIMATES; VISCERAL LEISHMANIASIS; VECTOR SURVEILLANCE

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P2-105: ECLIPSE RESEARCH PROJECT - CUTANEOUS LEISHMANIASIS AND STIGMATIZATION PROCESSES. A QUALITATIVE ANALYSIS IN ENDEMIC UNDERSERVED RURAL COMMUNITIES OF THE SOUTH OF BAHIA, BRAZIL

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Stigma is highly associated with the experience of cutaneous leishmaniasis (CL), but its analysis is not well reported by the international literature. This study is part of the 4 years international and interdisciplinary research project ECLIPSE - Empowering people with Cutaneous Leishmaniasis: Intervention Programme to improve patient journey and reduce Stigma via community Education. This work uses qualitative and quantitative methodologies to study CL and has a strong community engagement and involvement focus. Between 2020 and 2022 we performed ethnographic fieldwork, participant observation and 75 unstructured interviews with people with differing experiences of CL – community members, managers and professionals of local health and education services, volunteers of CL injection application and traditional healers - in CL hyperendemic rural areas of Bahia State in Brazil. We used NVIVO[R] software for thematic analysis content based on an intercultural and comprehensive health approach. The results provide evidence of the complexity of the stigmatization processes expressed by the participants' perceptions. Even if there is a normalization process linked with the resistance and resilience to CL in affected communities, we find various experiences of stigma at all the

social levels – individual, familiar, community - in the rural or urban contexts. While these experiences were common, some determinants, including age, race and residential area, led to greater vulnerability to stigma. There was greater evidence of stigmatization for black people, children and adolescents, women and rural area inhabitants. In these areas, CL stigma is generated by the visual perception of the ulcers, wounds and scars and olfactive perception of their "nasty smell". Moreover, outside the "comfort zone" of the rural communities – in the urban areas or in the healthcare services for CL treatment - the impact of the CL stigma is considered more impactful on their life as well as more complex to understand. This because CL stigma is compounded by other stigmas suffered by the members of the endemic rural communities defined as: "communities living next to the dumping ground", "dirty" and "poor" people, leaving in communities considered as "CL carriers", leaving "in the place where CL leaves". In both social contexts, the rural and urban areas, people affected by CL feel emotions like "shame", "fear" and "preoccupation" for the preconceptions of "the other'' in various situations and social places and times – school, public transport, public places and services. This causes self and social stigmatization that leads to direct social and economic consequences, e.g., self-isolation, school and work interruption or discontinuity. Local interventions including health education and information on the experience of CL are needed to improve the awareness and cultural sensibility against CL stigma in these territories characterized by the endemic precarity of these underserved rural communities. To improve quality of life and reduce CL sociocultural and mental health high impacts, local policies must be informed by this evidence. The ECLIPSE project is now acting with the engagement and involvement of the local actors to reach these goals.

Keywords STIGMA; CUTANEOUS LEISHMANIASIS; RURAL COMMUNITY; AWARENESS; BRAZIL

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P2-106: INTERSECTORAL TRAINING AND COMMUNITY ENGAGEMENT IN CUTANEOUS LEISHMANIASIS' ENDEMIC RUAL TERRITORIES IN SOUTH BAHIA, BRAZIL. THE ECLIPSE EXPERIENCE

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The study analyses group training courses to orient knowledge, practices and health services organization for the care of people affected by Cutaneous Leishmaniasis (CL). The training was structured in a intersectoral health approach and based on collaborative teaching practices and experiences sharing. This is a qualitative research-intervention study, based on the education and professional training practices. The territorial focus is a CL endemic area in the municipality of Valença, South of Bahia, Brazil. It is part of a research project "ECLIPSE - Empowering people with Cutaneous Leishmaniasis", a multisite research and intervention project that aims to improve care itineraries and reduce stigma through community education and engagement, that is realized in three countries - Sri Lanka, Ethiopia and Brazil, coordinated by UK's academic research institutions. We analyse two training courses realized between august 2021 and march 2022 with community members, professionals and managers of local education and healthcare services. Eighty-six persons were enrolled and sixty effectively participated. In the first workshop the participants were mostly the professionals of "rural education" and some community members, while in the second one enrolled only health managers and professionals - mainly nurses - of the urban area and also some community health workers of the rural endemic areas. The educational approach adopted the PBL - Problem-Based Learning pedagogy model for a heterogeneous target group of public workers in order to multiply the information on CL in the rural communities.

The proposal of this methodology was produced by previous group discussion and planning meetings led by three ECLIPSE Brazil team researchers. Six different workshops were defined for a total of 20 hours, focusing on the following dimensions of CL: 1. The clinical forms, diagnosis and treatment of CL; 2. Eco-epidemiological CL cycles; 3. Etiological agent and vector of CL; 4. Cultural dimensions in endemic communities; 5. Sociocultural determinants of CL; 6. CL Prevention and control strategies. Due to the pandemics of COVID19, the first edition of the training course was realized entirely virtually (on-line meetings and off-line remote activities), using Zoom^{R} platform, while in the second one 2/3 of the meetings were face-to-face. The engagement and participation were sensibly higher in the face-to-face meetings, also because of the low internet accessibility in the rural areas. In each workshop most of the participants were black women, health professionals, with high education level, residents of the urban area, and even if a small group had experienced CL, the majority knows people affected by the disease. We evaluated with quali-quantitative instruments the low level of knowledge about CL at the start of the training, in both editions. The final evaluation of the workshops pointed out the growing awareness on CL and that all the participants evaluate useful for their professional work in the rural communities, and the satisfaction for the quality of the training was high. We recommend this model of training workshops based on the dialogue between different knowledge and experiences as a strategy to enhance professional awareness and to guarantee community engagement and participation against CL.

Keywords HEALTH HUMAN RESOURCE; COMMUNITY ENGAGEMENT; INTERSECTORIALITY; CUTANEOUS LEISHMANIASIS

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P2-107: FOOD AND NUTRITIONAL DIALOGUES ON THE EXPERIENCES OF CUTANEOUS LEISHMANIASIS: KNOWLEDGE GAPS, SOCIOECONOMIC AND CULTURAL ASPECTS

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This study aims to present and analyse aspects related to food and nutrition of people affected by cutaneous leishmaniasis (CL) in an endemic area. The importance of these issues has been reported by the studies focusing on the evolution of nutritional status in people with CL, the relationship between dietary pattern, clinical and therapeutic evolution of the disease, the effects on the immune system, especially in the contexts of nutritional deficiency and malnutrition. Cultural issues and food insecurity dimensions are lacking. In Brazil, CL impacts mainly indigenous and black populations, health with high socioeconomic vulnerability. The success of actions to face CL depends on the understanding of these vulnerable contexts, the availability of intersectoral actions to create healthcare networks and the perception of the knowledge and practices of these populations. Other important issues are the food and nutritional culture, the population's empirical knowledge of the relationship between food and the clinical evolution of the disease. These questions emerged from the ethnographic insigths carried out in rural communities of an endemic area for *CL* in Brazil. This study is part of "ECLIPSE-Empowering people with Cutaneous Leishmaniasis", an international research and intervention project developed in three countries: Sri Lanka, Ethiopia and Brazil, coordinated by UK's academic research institutions. The narratives produced in the community and health services about experiences with CL, highlighted issues related to food and nutrition based on local knowledge, as well as doubts and insecurities about the most appropriate diet during medical

treatment. The empirical knowledge of rural communities refers to a selection of foods that affect the inflammatory process of lesions, delaying the healing process or generate discomfort because of their perceptible smell. Some aliments are considered unsuitable for consumption during the disease, such as rice, seafood, shellfish, chicken, pork, among others. These correlations are not proposed by all people with CL experience. In medical literature, there is no evidence of specific dietary restrictions in cases of CL and in fact the medical advice recommends only healthy diet. This orientation does not consider the cultural norms that orient the local diets in case of CL nor the socioeconomic dimensions of the communities. There is no test of the physiopathological action of these foods, but empirical perceptions highlight the need to extend these studies. Moreover, the medical recommendations are inefficient in a context where there are situations of food insecurity, malnutrition and hunger. Food insecurity and the cultural can accentuate the disease' suffering. We recommend defining the policies and action for the promotion, prevention and care starting from the effective participation of the communities, to developpe collective prophylactic approaches and singular therapeutic projects. In addition to drug treatment, food and nutrition is a major factor for the recovery of health conditions and requires actions to ensure the human right to adequate food and nutritional food security.

Keywords CUTANEOUS LEISHMANIASIS; SOCIAL VULNERABILITY; CULTURAL CHARACTERISTICS; FOOD AND NUTRITION SECURITY

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P2-109: CONCEPTIONS AND PRACTICES ABOUT LEISHMANIASIS OF THE AWÁ, EMBERÁ AND MURUI PEOPLES OF PUTUMAYO: AN EXAMINATION OF HEALTH/DISEASE/CARE THROUGH THEIR OWN MEDICINE

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The department of Putumayo is located in southern Colombia. According to the calculations of the Colombian National Administrative Department of Statistics (DANE), it has 359,127 inhabitants, of which 176,841 (49.25%) live in village communities and scattered rural areas. It contains fifteen indigenous groups that represent 17.9% (50,694) of the total population. This cultural diversity embraces at least 15 different ways of understanding the health/disease/care triad. Although different indigenous peoples may live in the same territory, the ways of interacting with the land and conceiving it vary; this also happens in the particular case of leishmaniasis. Putumayo is hyperendemic for cutaneous leishmaniasis, because the vector, the parasite, and the natural and cultural conditions are all present, such that cases occur throughout the year. The members of the Awá, Emberá and Murui communities all recognize the disease, but identify different causes and treatments, for example. Each indigenous people has a word for leishmaniasis: *Guaral* (Awá); *Pithw* or *Pito* (Emberá); and *chik+ etaibaki* or *Pito* (Murui). For the Awá, leishmaniasis is caused by *Guaral*, a plant that grows on trees and stones, and next to streams: "it sticks to vines … when the bush is cut, it releases a milky substance, and if a person touches the plant, it infects them … there is a male and female *Guaral*." For the Emberá communities, it is caused by an insect bite; contact occurs when they work in their traditional cultivation spaces or in their houses. For the Murui

peoples, the disease breaks out when the armadillo is close to the community and, with it, comes the *Pito*. The nocturnal curassow is also believed to attract mosquitoes, which is why Murui elders recommend not imitating curassow sounds so that mosquitos don't come to the house. The Awá classify leishmaniasis as a disease specific to their people, one that is transmitted by a plant. For the Murui, it is a disease produced by a mosquito, but not associated with the Lutzomyia genus, known to them as *Aziza+* or *Manta Blanca*. The Emberá communities do correlate Lutzomyia, which they call *Emu*, with leishmaniasis. According to the Awá peoples, cases arise due to the disobedience of people who enter sacred sites and have contact with *Guaral*. The Murui believe that cases occur due to lack of prevention, and the Emberá associate cases with the appearance of the new moon. Each therapeutic system promotes prevention, control and care actions in accordance with their uses and traditions. Actions may include discussions regarding healthcare in the *mambeos* (traditional ritual spaces), ceremonies to drive away insects, use of incense in homes, and treatments with medicinal plants provided by traditional knowledge-keepers. This latter group directly attends to and controls cases of disease in the communities; they all agree on the need to provide a cure, since disease generates disharmony in the individual, community, and territory. Coordinated efforts with western healthcare teams toward the prevention of leishmaniasis have yet to be achieved.

Keywords TRADITIONAL MEDICINE; INDIGENOUS PEOPLES; PUTUMAYO; LEISHMANIASIS; BELIEFS; PRACTICES

Financing Amazon Conservation Team (ACT)

P2-110: BARRIERS TO DIAGNOSIS AND CONTROL OF LEISHMANIASIS IN PUTUMAYO, COLOMBIA: INSTITUTIONAL EXPERIENCE WITH VECTOR-BORNE DISEASES (VBD)

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The department of Putumayo is located in southern Colombia. It has an area of 27,820 km^2, which represents 2.26% of the national territory. Nearly 85% of its territory belongs to the Amazon Plains landscape unit. According to the calculations of the Colombian National Administrative Department of Statistics (DANE), it has 359,127 inhabitants, with 182,286 (50.75%) distributed in the urban area, and 176,841 (49.25%) distributed across village communities and dispersed rural areas. This department is hyperendemic for cutaneous leishmaniasis, because the vector, the parasite and the natural and cultural conditions are all present so that cases occur throughout the year. Per public health records in the department, between 2007 and 2021, 4,496 cases of leishmaniasis (4,439 cutaneous and 57 mucosal) were reported. The most affected subregions are the Middle and Lower Putumayo. The majority of reports originate in 7 of the department's 13 municipalities, the most affected being Puerto Asís, Puerto Guzmán, Puerto Leguizamo, Orito, Puerto Caicedo and Villagarzón. Agents responsible for vector-borne diseases (VBD) have traveled the territory with different strategies for disease diagnosis and control. They have encountered four primary barriers that have hindered work toward eradication or reduction of the disease: 1. Geography: the distances between hospitals and communities are significant; there are insufficient communication channels; and a large number of the communities are accessible only by river or air. 2. Lack of security: access to diagnosis and treatment may be limited by security and public order conditions, and patients may prefer to remain in their territory so as not to expose

themselves. In addition, the fieldwork of the VBD personnel (active search, prevention, and physical control of the vector, among others) is also impacted by those engaged in the conflict, limiting access to afflicted communities. 3. Medication: Glucantime® causes patients to abandon treatment due to strong side effects, and patients may resort to indigenous medicine. Others may choose to self-medicate with aggressive treatments such as gunpowder, cigarette ash, drops of sulfuric in combination with plants, and bicarbonate, among others, which may leave serious skin lesions and will fail to cure the disease. Finally, those who live in dispersed rural areas and decide to continue treatment with Glucantime® face an economic barrier to access: the distribution of this drug is centralized, and they may not have sufficient resources to travel multiple times to urban areas to receive the treatment. 4. Lack of coordination at the institutional level: there is insufficient coordination between health-promoting entities, hospitals, and departmental and municipal health secretariats for patient care in the rural and dispersed rural areas. To address this disease in such a complex context, intersectional strategies that engage the affected communities are required. Additionally, a change in the focus of the healthcare model is needed, entailing more institutional support for patients as well as guarantees with respect to security in order to achieve access to diagnosis and treatment.

Keywords PUTUMAYO; CUTANEOUS LEISHMANIASIS; BARRIERS; DIAGNOSIS, CARE

P2-111: FINANCIAL AND ADMINISTRATIVE CHALLENGES IN IMPLEMENTATION OF CLINICAL TRIALS IN RESOURCE LIMITED SETTINGS: EXPERIENCES FROM DNDI SPONSORED CLINICAL TRIALS IN EASTERN AFRICA

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Conducting clinical trials (CT) requires adequate resources and administrative support to ensure success. Trialists focus more on the scientific aspects of the clinical trials, yet financial resources and administrative support are also key drivers that guarantee successful completion of the clinical trials, especially in resource limited settings. To describe the financial and administrative challenges experienced in the conduct of clinical trials in resource limited settings in Africa and provide recommendations that may have a positive impact in the future of clinical trials conduct. In the last fourteen years we have conducted numerous trainings (5 workshops and several one-on-one sessions) on Good Financial Practice (GFP), documented the lessons learned and challenges encountered. Qualitative data on key success factors and challenges of conducting clinical trials was collected mainly through observations, case studies and literature review and analyzed. Data was extracted from the CTs reports, financial reports, site visits by the finance leaders and communications with the partners, especially the investigators and the Finance (sponsor) leader. Additionally, experiences encountered during contract negotiations with partners participating in clinical trials sponsored by DNDi in eastern Africa have provided key information towards this endeavor. Major financial and administrative challenges identified in CT implementation include poor budgeting techniques, inadequate negotiation skills, bureaucratic systems, poor infrastructure (roads and internet connectivity), high staff turnover at trial sites, lack of diligence during review of clinical trial agreements before appending signatures, lack of

partner finance staff understanding of CT needs and inadequate financial management and reporting skills by CT sites. Key highlight is late disbursement of trial funds by sponsors due to amongst other reasons, studies startup delay, sites not meeting contract requirements and/or political challenges that impair financial transactions. Financial and administrative processes are key in ensuring clinical trials objectives are successfully achieved. Identifying the right skillsets, dedicating specific HR at the trial sites to deal handle financial and administrative issues, and continuously training key partner finance personnel in financial administration can address the identified challenges and expedite clinical trials conduct. Positioning GFP the way GCP has been positioned globally making it a requirement for key clinical trials personnel to undergo a refresher GFP training at the start of each study would also provide solutions to the identified challenges. There is need to rethink partnership and advocacy to target other actors (eg customs and revenue authorities, courier companies) who are involved in the supply chain process to facilitate delivery of trial commodities.

5.8 VECTORS & RESERVOIRS

P1-106: INVESTIGATION OF NATURAL INFECTION OF PHLEBOTOMINE (DIPTERA: PSYCHODIDAE) BY *Leishmania* IN TUNISIAN ENDEMIC REGIONS

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Leishmaniases are caused by protozoan parasites of the genus Leishmania transmitted by females blood-feeding phlebotomine insects (Diptera: Psychodidae). In Tunisia, cutaneous and visceral leishmaniases are of public health concern. In Tunisia, 17 species of phlebotomine sand flies are described. Here we investigate natural infection in Tunisian mixed foci regions of leishmaniases. We trap female sandflies during the *Leishmania* transmission season in the country's central-eastern and northern parts. We investigate *Leishmania* infection using PCR-RFLP targeting the ITS1 ribosomal DNA, followed by enzymatic digestion with HaeIII; then, we identify sand flies using molecular methodologies. We confirm the presence of *Phlebotomus papatasi* and *Phlebotomus perniciosus* infected by *L. major* and *L. infantum* parasites in Tunisia.

P1-107: GENE EXPRESSION OF ANTIMICROBIAL PEPTIDES IN *Phlebotomus papatasi* REVEALS A GUT-SPECIFIC DEFENSIN UPREGULATED BY *Leishmania major* INFECTION

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The Leishmania parasites develop within a diverse microbial environment in the sand fly gut. At the same time, the sand fly immunity responds to this complex microbiota to maintain its balance. In the present work, we have chosen *Phlebotomus papatasi*, the natural vector of *Leishmania major*, and aimed to address two specific questions. a) Is the sand fly immune response triggered by *Leishmania major* or bacteria? b) Is this response tissue-specific? We investigated the expression of genes coding for molecules involved in two of the pathways that regulate the innate insect immunity. We selected the transcription factors dorsal and relish belonging to the Toll and immune deficiency (IMD) pathways, respectively. In addition, we investigated two antimicrobial peptides (AMPs), attacin and defensin. All gene sequences were identified by similarity with other previously identified insect sequences. First, we studied sand fly larvae, having the experimental group fed with microbe-rich food and the control group fed with autoclaved food. Larvae samples were collected at different stages (L2, L3, early-L4, and late-L4). Total RNA from larvae was used for cDNA synthesis, followed by relative gene expression analysis (qPCR). In the group fed with microbe-rich food, we observed an increased expression of relish, defensin, and attacin in various larval stages. The increase in AMPs expression resulted from the microbe-rich regimen and may control the extra load of ingested bacteria. In the second series of experiments, we used adult females. We depleted their gut bacteria by antibiotic cocktail (AtbC) treatment and infected them with *L. major*. One infected group was constantly treated with AtbC. In contrast, the second infected group had the AtbC treatment removed, thus allowing bacteria to regrow in the sand fly

gut. A control group was constantly treated with AtbC and fed with blood without the parasites. Samples were collected at 24h, 48h, 72h, and 144h post-infection and analyzed as described for larvae. The defensin gene was upregulated six days post-infection in the *Leishmania*-infected group with depleted gut bacteria and three days post-infection in sand fly females with recovered gut bacteria. Results indicate that this gene was induced by both *L. major* and the regrown bacteria. Interestingly, this defensin was expressed only in dissected guts, indicating a tissue-specificity. The identification of a defensin gene upregulated by *L. major* infection is important for future genetic-based strategies to control the parasite transmission. We are currently investigating the role of defensin and other immune-related genes by RNAi-mediated gene silencing to characterize their function.

Keywords SAND FLY; IMMUNE RESPONSE; TISSUE-SPECIFIC; GENE EXPRESSION

Financing This study was supported by the Czech Science Foundation (GACR21-15700S), Grant Agency of Charles University (1380120) and ERD funds, project CePaViP (16_019/0000759).

P1-108: MOLECULAR ASSESSMENT OF BLOOD MEAL SOURCE IN FIELD-CAPTURED SAND FLIES (DIPTERA: PSYCHODIDAE) IN ROMANIA

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Blood feeding preferences of sand fly species on vertebrate hosts are risk indicators of potential new foci of human and animal leishmaniosis. In the present study, the blood meal source of engorged females of *Phlebotomus perfiliewi* and *Ph. neglectus*, captured in Romania between 2013 and 2021, was molecularly assessed using a previously described protocol. Only engorged sand fly females (n = 74) were tested. In total, 52/74 (70.3%) sequences were obtained, 32/52 (61.5%) for *Ph. neglectus* and 20/52 (38.5%) for *Ph. perfiliewi*. In *Ph. neglectus,* 1/32 (3.1%) fed on *Apodemus agrarius*, 7/32 (21.9%) on *Cervus elaphus*, 7/32 (21.9%) on *Homo sapiens*, 3/32 (9.4%) on *Lepus europaeus,* 11/32 (34.4%) on *Bos taurus*, 2/32 (6.2%) on *Capreolus capreolus* and 1/32 (3.1%) on *Sus scrofa*. In *Ph. perfiliewi*, 1/20 (5.0%) fed on *Bos taurus*, 1/20 (5.0%) on *Ovis aries*, 1/20 (5.0%) on *Gallus gallus*, 16/20 (80.0%) on *Equus caballus* and 1/20 (5.0%) on *Homo sapiens*. The various domestic and wild vertebrate species may indicate the opportunistic feeding behavior of the sand fly species increasing the risk of disease transmission between domestic, peri-domestic and sylvatic reservoirs of public health concern. This is the first molecular assessment of blood meal source in field-captured sand flies in Romania. The results cannot be generalized to the entire Romanian territory, nor to all sand fly species present in Romania. More studies are necessary for a better

understanding of each sand fly species feeding behavior and for the possible risk transmition of human and animal leishmaniasis.

Keywords *Phlebotomus perfiliewi; Ph. Neglectus; b*LOOD FED; SAND FLIES, COX1, ROMANIA

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P1-109: REPEATED SAND FLY BITES OF INFECTED BALB/C MICE ENHANCE THE DEVELOPMENT OF *Leishmania* LESIONS

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Sand fly saliva has immunomodulatory effect on the course of *Leishmania* infection in mammalian host. Several studies have been done on *Leishmania* – sand fly – host model, which show that pre-exposure of the hosts to uninfected sand fly bites provided significant protection against infection, while co-inoculation of *Leishmania* parasites with salivary glands lysates or salivary peptides enhanced pathogenicity. The third scenario, the effect of the sand fly saliva on parasite infection in host infected before exposure to sand flies was the main aim of the study to better understudied *Leishmania* – sand fly – host interactions. BALB/c mice were intradermally infected with sand fly-delivered *L. major* parasites and divided into two groups. First group were repeatedly bitten by *Phlebotomus duboscqi* females every two weeks and second group was left as unexposed control. Course of infection was monitored weekly. The parasite load and distribution in the viscera were tested *post-mortem* using qPCR. Repeated sand fly bites significantly affected the development of cutaneous lesions. In mice exposed sand flies, lesions developed faster and reached larger than in unexposed mice. Multiple sand fly bites also increased parasites load in inoculated ears, but distribution of parasites in mice body and their infectivity for vectors did not differ between the experimental groups. These results prove that multiple and repeated exposures of infected BALB/c mice to *P. duboscqi* females significantly enhance the progress of local skin infection caused by *L. major* and increase tissue parasite load, but do not effects on the visceralization of parasites.

P1-110: HERITABLE SYMBIONTS AND MICROBIOTA DIVERSITY ANALYSIS IN PHLEBOTOMINAE SAND FLIES AND *Culex nigripalpus* FROM COLOMBIA

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Secondary symbionts of insects include a range of bacteria and fungi that perform various functional roles on their hosts, such as fitness, tolerance to heat stress, susceptibility to insecticides and effects on reproduction. These endosymbionts could have the potential to shape microbial communites and high potential to develop strategies for mosquito-borne disease control. Different percentages of natural infection by *Wolbachia, Cardinium, Arsenophonus* and *Microsporidia* in phlebotomines and mosquitoes from Colombia were detected. High percentages of relative abundance for *Wolbachia* in *Lu. gomezi, Ev. dubitans, Mi. micropyga, Br. hamata,* and *Cx. nigripalpus* were found. ASVs assigned as Microsporidia were found in greater abundance in *Pi. pia* and *Cx. nigripalpus*. An important finding is the detection of *Rickettsia* in *Pi. pia* and *Bartonella sp.* in *Cx. nigripalpus.* he microbiota profiles of Sand flies and mosquitoes showed mainly at the phylum level to Proteobacteria (67.6%), Firmicutes (17.9%) and Actinobacteria (7.4%). The Principal Coordinate Analysis (PCoA) is consistent, which showed statistically significant differences (PERMANOVA, F = 2.4744; R2 = 0.18363; p-value = 0.007) between the microbiota of sand flies and mosquitoes depending on its origin, host and possibly for the abundance of some endosymbionts (*Wolbachia, Rickettsia*). Specifically, this study determined the identity and phylogenetic location of some strains of these endosymbionts. Also, it was possible to determine

whether Wolbachia abundance and insect species are important in the patterns of the microbiota.

Keywords SECONDARY SYMBIONTS; *Wolbachia; Rickettsia; Bartonella* sp*;* SAND FLIES; COLOMBIA.

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P1-111: GUT MICROBIOTA DYNAMICS IN NATURAL POPULATIONS OF *Pintomyia evansi* UNDER EXPERIMENTAL INFECTION WITH *Leishmania infantum*

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Pintomyia evansi is recognized by its vectorial competence in the transmission of parasites that cause fatal visceral leishmaniasis in rural and urban environments of the Caribbean coast of Colombia. The effect on and the variation of the gut microbiota in female *P. evansi* infected with *Leishmania infantum* were evaluated under experimental conditions using 16S rRNA Illumina MiSeq sequencing. In the coinfection assay with *L. infantum*, 96.8% of the midgut microbial population was composed mainly of Proteobacteria (71.0%), followed by Cyanobacteria (20.4%), Actinobacteria (2.7%), and Firmicutes (2.7%). In insect controls (uninfected with *L. infantum*) that were treated or not with antibiotics, *Ralstonia* was reported to have high relative abundance (55.1-64.8%), in contrast to guts with a high load of infection from *L. infantum* (23.4-35.9%). ASVs that moderately increased in guts infected with *Leishmania* were *Bacillus* and *Aeromonas*. Kruskal-Wallis nonparametric variance statistical inference showed statistically significant intergroup differences in the guts of *P. evansi* infected and uninfected with *L. infantum* ($p < 0.05$), suggesting that some individuals of the microbiota could induce or restrict *Leishmania* infection. This assay also showed a

negative effect of the antibiotic treatment and *L. infantum* infection on the gut microbiota diversity. Endosymbionts, such as Microsporidia infections (<2%), were more often associated with guts without *Leishmania* infection, whereas *Arsenophonus* was only found in guts with a high load of *Leishmania* infection and treated with antibiotics.

Keywords *Arsenophonus*; *Pintomyia evansi*; *Ralstonia*; MICROBIOTA; MICROSPORIDIA

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P1-112: POTENTIAL VECTORS OF *Leishmania infantum (L. (L.) infantum chagasi)* IN SANTA CRUZ DO SUL, RIO GRANDE DO SUL, SOUTH OF BRAZIL

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American Visceral Leishmaniasis (AVL) is an emerging disease in the state of Rio Grande do Sul, southern Brazil. In this state, there is a pattern of transmission different from the predominant in the country, where the main vector is *Lutzomyia longipalpis*. Although this species can be found in the west of the state near the border with Argentina, there are endemic areas in cities of the east (Porto Alegre and Viamão) and central region (Santa Cruz do Sul), where this vector was not detected by the entomological surveillance. Santa Cruz do Sul is the city where this pattern has been happening for the longest time in the state. Since 2004, visceral leishmaniasis canine cases have been reported every year in its urban area, although there is no report of human cases. The aim of this study is to identify the sand fly fauna in transmission areas of Santa Cruz do Sul to verify the presence of *Lu. longipalpis* and other potential vectors, providing information about the ecoepidemiology of AVL in the south of Brazil. Five domiciles classified as probable site of infection of canine cases were selected. In each location, one HP light trap was set in places propitious to sand flies, located within a radius of up to 50 meters from the domicile. The captures were performed during three nights per month from January to December of 2020. Additionally, Castro aspirators were used to capture sand flies resting on the walls and animal shelters of each domicile at night and a Shannon trap was used in one sampling site from 18:00 to 22:00 hours, once a month. A total of 381 sand flies belonging to six genera were

captured. The species were *Brumptomyia* spp. (39,11%), *Migonemyia migonei* (21,26%), *Pintomyia fischeri* (20,21%), *Evandromyia edwarsi* (10,24%), *Psathyromyia lanei* (4,99%), *Martinsmyia alphabetica* (1,84%), *Psathyromyia pascalei* (0,79%) and *Evandromyia gaucha* (0,52%). The most abundant species of medical importance were *Mg. migonei* e *Pi. fischeri* and were the only species caught in all sampling sites. Both were recently incriminated as putative vectors of *Leishmania infantum chagasi* in Brazil and have wide distribution in the country. In Porto Alegre, capital of the state, DNA of *L. infantum chagasi* was detected in pools of *Pi. fisheri* and *Mg. migonei* captured near human cases supporting their possible role as vectors. These species might also be involved in the AVL transmission cycle in Santa Cruz do Sul, since *Lu. longipalpis* still not found. However, further studies are necessary to elucidate their epidemiological role. In Brazil, the presence of *Lu. longipalpis* is used by the surveillance to classify receptive and vulnerable areas. The alleged absence of this species in areas of canine or human cases, combined with new studies on putative vectors, alerts to the need for change in the brazilian strategies of AVL surveillance.

Keywords VISCERAL LEISHMANIASIS; VECTOR; SURVEILLANCE; SAND FLY

Financing Instituto Oswaldo Cruz, Secretaria da Saúde do Rio Grande do Sul

P1-113: SOCIO-ENVIRONMENTAL FACTORS ASSOCIATED WITH THE OCCURRENCE OF *Lutzomyia longipalpis* IN ENDEMIC AREA OF VISCERAL LEISHMANIASIS IN NORTHEASTERN BRAZIL

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Lutzomyia longipalpis is incriminated as the main vector of *Leishmania (L.) infantum chagasi*, the *Leishmania* sp. responsible for American Visceral Leishmaniasis (AVL) in Brazil, which concentrates about 96% of AVL cases in the Americas, with the Northeast Region the most affected with 49% of reported cases in the country. Poverty and socio-environmental conditions are identified as the main factors for prevalence for this disease, however outbreaks in Brazilian urban centers have been reported and associated with urbanization. The municipality of João Pessoa, capital of the state of Paraíba, Northeast Brazil, is classified as an area of intense transmission of AVL, continuously notifying cases of the disease in humans and dogs. The objective of this study was to verify the association among socio-environmental variables and the occurrence of *L. longipalpis* in the municipality of João Pessoa. HP-type light traps were installed in the peridomicile area of the residences in urban area of the municipality from May/2019 to March/2020, August/2020 to February/2021 and March to July/2021. A Generalized Linear Model (GLM) was constructed using as response variable the abundance of *L. longipalpis* and as predictors variables temperature and average humidity, number of people in the house, income, presence of a paved street, sewage system, fruit trees, chickens and dogs. A total of 121 residences were sampled, distributed in 42 neighborhoods (60% of the total municipality) during 4 consecutive days (3 nights). The vector *L. longipalpis* was captured in 28 residences, distributed in 20 neighborhoods of the city, representing 47.6% of the sampled area and 28.6% of the total city. According to the GLM model, presence of a paved

street (estimate=-1.8615, p=0.0008) and temperature (estimate=-0.3278, p=0.0331) had a negative and significant association with the abundance of *L. longiplapis*, while the presence of chickens showed a significant and positive association (estimate=1.1442, p=0.0287). The variable paved street implied a negative association with the abundance of *L. longipalpis*, probably due the fact that the pavement provided a decrease in the accumulation of organic matter, hindering the development of immature forms. In addition, the concrete used in the pavement favors the increase of the local temperature and, as shown in the model, the abundance of the vector is related to lower temperatures. Otherwise, the presence of chickens is related to an increase in the abundance of the vector, since the presence of these animals causes accumulation of local organic matter, favoring the development of sandflies, and the possibility of females using chickens as blood source. Therefore, government actions are necessary, aiming at improving infrastructure conditions and, above all, health surveillance actions in order to sensitize the population about the need for environmental management in the creation of domestic animals, in addition to maintaining a constant vector monitoring program of *L. longipalpis* in areas of human and canine transmission.

Keywords *Lutzomyia longipalpis*; SURVEILLANCE AND CONTROL; AMERICAN VISCERAL LEISHMANIASIS; NORTHEAST BRAZIL

Financing State Health Department of João Pessoa and Instituto Oswaldo Cruz/ FIOCRUZ

P1-115: NEW REPORTS OF PHLEBOTOMINE FAUNA AND THEIR INFECTION WITH DIFFERENT SPECIES OF *Leishmania* spp. IN THE MBARACAYU FOREST BIOSPHERE RESERVE, PARAGUAY

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Leishmaniasis is a group of vector-borne diseases belonging to the family Phlebotominae and caused by different species of the genus *Leishmania* spp. In Paraguay, leishmaniasis, in all its forms, is a public health problem. Each year there are a significant number of cases of cutaneous forms and a significant increase in cases of the visceral form of the disease in urban and ruro-urban areas. The purpose of this study was to determine the abundance of sandflies in an area of the Mbaracayú Forest Biosphere Reserve (RBBM in Spanish), Canindeyú Department (Paraguay) and to determine the natural infection rate of *Leishmania* spp. in the sandfly population by PCR-HRM DNA amplification. Three zones of the RBBM with different degrees of environmental degradation were the study area. The capture of sandflies was performed with 10 CDC type light traps, in three sentinel sites during two consecutive nights, once a month from October 2020 to 2021. DNA extraction and purification from female sandflies was performed using the Gene JET Genomic DNA Purification Kit® following the manufacturer's instructions. To confirm the presence of *Leishmania* spp, a fragment of ITS1 gene was amplified using the primers described by de Almeida et al. (2016). Identification of sandfly species was performed *in situ* using the key of Galati 2003 and abbreviations according to Marcondes 2007. A total of 1129 sandflies were captured, 571 females and 378 males, and 11 species were identified. The most frequent species were: *Evandromyia cortelezzi* complex (27.7%), followed by *Brumptomyia brumpti*

(25.4%), *Pintomyia montícola* (12.7%), *Psathyromyia lanei* (3.7%), *Nyssomyia neivai* (2.5%), Evandromya evandroi (1.5%), *Brumptomyia guimaraesi* (0.6%), *Micropygomyia quinquefer* (0.5%), *Evandromyia termitophila* (0.3%), *Migonemia migonei* (0.2%), *Psathyromyia shannoni* (0.2%) (pending classification, 25%). A total of 531 females were molecularly processed and 14% were positive for *Leishmania* spp, of which 46% corresponded to *L. infantum*, 22% to *L. amazonensis* and 32% to *Leishmania* spp. The species with presence of *Leishmania* spp DNA were *B. brumpti, Ev. cortelezzi complex, Ev. evandroi, Ny. neivai, Pi. montícola* and *Pa. lanei.* The presence of *L. infantum* and *L. amazonensis* was detected in specimens of *B. brumpti* and also in *Pi. montícola*. Nine species of sandflies are recorded for the first time for the Department of Canindeyú, as only *B. brumpti* and *Mg. migonei* were previously documented. This is also the first report of the presence of *Ev. cortelezzi* in a forested area. Although no *Lutzomyia longipalpis* was captured, four species had L*. infantum* DNA. This is the first report of the presence of DNA from *L. amazonensis* in *B. brumpti* and *Pi. monticola* in Paraguay. The presence of different *Leishmania spp.* DNA in six phlebotomine sandfly species in the RNBM, a reserve surrounded by settlements and an important tourist site, shows the high potential risk of leishmaniasis transmission in places with different degrees of environmental degradation.

Keywords PHLEBOTOMINAE, *L. infantum, L. amazonensis*, PCR-HRM, PARAGUAY

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P1-116: FOURTH LARVAL INSTAR CHARACTERS FOR THE DIFFERENTIATION OF SPECIES IN THE SUBGENUS *Pifanomyia* BASED ON THE DESCRIPTION OF *Pintomyia longiflocosa*

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The study of larval morphology of Phlebotominae sand flies has been limited by the difficulty in identifying natural breeding sites and in laboratory rearing. Nonetheless, the chaetotaxy of immature stages is of great interest in the systematics of the subgenus *Pifanomyia* and the fourth larval instar of *Pintomyia serrana, P. youngi, P. ovallesi, P. verrucarum and P. evansi* have been partially or totally described. *Pintomyia* (*Pifanomyia*) *longiflocosa* is an endemic species from Colombia found between the Central and Eastern Andes from 900 to 2100 m.a.s.l. It has been reported as one of the primary vectors of cutaneous leishmaniasis in coffee growing zones of the country. This species is classified among the Townsendi series and can only be identified by the morphology of the male adults. As an alternative to identification in cryptic species the study of the morphology of immature stages has shown potential in the discovery of new useful taxonomic characters. We therefore described the chaetotaxy of the fourth larval instar of *P. longiflocosa* and compared it to previous descriptions of closely related species. *Pintomyia longiflocosa* adults were captured in Campoalegre, Huila and reared in the Entomology Laboratory at the Instituto Nacional de Salud de Colombia. To identify the setae found in each corporal segment a total of 15 fourth instar larvae were mounted on microscope slides using Canadian Balsam after being cleared with potassium hydroxide (KOH 10%) and saturated phenol. Additionally, 5 specimens were prepared for observation under Scanning Electron Microscopy (SEM). A series of illustrations showing the position and relative size of each structure were obtained based

on the observations made on the microscopic mounts and the SEM images. Using the chaetotaxy nomenclature system proposed by Forattini (1973) common patterns were found in the head and thorax among the species in the *Pifanomyia* subgenus. However, presence or absence of certain setae suggest differences in the fourth larval instar of this group of species. Specifically, in the Townsendi series it was possible to identify differences with *P. youngi*, the only previously described fourth larval instar of this series. Finally, when comparing *P. longiflocosa* with the available descriptions of larval instars of the subgenus *Pifanomyia*, all have the same antennal morphology, which allows a clear differentiation of this subgenus. These results support the potential importance of morphological characters from the fourth larval instar, such as antennal morphology and chaetotaxy, specifically in the case of closely related species that are cryptic in their adult stages. Furthermore, the shared patterns found among the species of *Pifanomyia* may be indicative of similar conditions in their breeding sites as the relative size of larval structures has been previously related with specific characteristics such as breeding site depth. The breeding sites of *P. longiflocosa* have not been explored, but the similarities found in the chaetotaxy could optimize future search based on the previous characterization of the microhabitat were the immature stages of *P. evansi*, *P. ovallesi* and *P. serrana* are found in Colombia.

Keywords CUTANEOUS LEISHMANIASIS; PHLEBOTOMINAE; PIFANOMYIA; LARVAE; CHAETOTAXY

P1-119: DIVERSITY AND SPATIO-TEMPORAL FREQUENCY OF SANDFLIES OF AN ARTIFICIAL WATER RESERVOIR IN A TROPICAL DRY FOREST (COLOMBIA)

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The rural population of tropical forests worldwide is exposed to Leishmaniasis, a neglected vector-borne disease transmitted by sandflies (Diptera: Psychodidae). Differences in the biology of vectors are closely related to several scenarios of Leishmaniasis risk. Then, to design effective control strategies, it is crucial to characterize the diversity of sandfly species and their relationship with their habitats. This characterization is essential for megadiverse countries where a rigorous taxonomic treatment of species is a challenging task. Here, we characterize the biodiversity of sandflies in the influence area of a relatively recently created artificial water reservoir, Tona, which is located in a tropical dry forest of northern Colombia –a megadiverse country–. Sampling was performed from 2017 to 2018, using HomeTrap-UIS, Torre Vigía-UIS, CDC, and *Bg-Sentinel* traps. Trap locations were selected considering the ecological features of the Tona reservoir, including the most preserved (pre-and post-Tona reservoir) and least preserved (intra-Tona reservoir) areas. We collected 286 samples, distributed in nine genera and 17 sandfly species, of which 14 were determined to species level. Here, we report three new records for Santander Department: *Evandromyia dubitans, Pintomyia youngi y Lutzomyia ceferinoi.* The most common species were *Pintomyia* sp. (15.7%) and *Pressatia camposi* (10.4%). Intra-Tona reservoir area presented the highest richness of species (16) and the largest number of samples (84.3%),

followed by post-Tona reservoir area (7 spp., 14.4% of samples). Most samples were collected with CDC trap (47.8%), followed by Torre Vigía-UIS (30.2%). Species collected in the Tona reservoir are of epidemiological relevance because of their anthropophilic nature. We suggest systematic entomological surveillance around influence area of the Tona reservoir, in particular for Intra-Tona reservoir area, to determine the risks of leishmaniasis transmission to the rural population of the area.

Keywords TAXONOMY; ENTOMOLOGY; *Pintomyia youngi; Evandromyia dubitans; Lutzomyia ceferinoi*

Financing Universidad Industrial de Santander.

P3-103: STANDARDIZATION OF BIOLOGICAL PARAMETERS FOR INSECTICIDE SUSCEPTIBILITY OF SAND FLIES

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Among the whole arsenal of strategies used in surveillance and control programs for vector-borne diseases, chemical control is the oldest tool that, in the long term, has already been shown to select populations of insecticide-resistant vectors. For the sandfly populations, which transmit leishmaniasis (visceral and tegumentary), the knowledge about the susceptibility of populations is essential, given the long period of insecticide' use. Also, recently, the Brazilian Ministry of Health started to use collars impregnated with insecticide as a tool for controlling visceral leishmaniasis, which could also impact on sandflies susceptibility. However, the methodology for performing bioassays in the laboratory has not been standardized for this insect group. The objective of this work was to define biological variables for the evaluation of *Lutzomyia longipalpis* susceptibility bioassays. Therefore, the variables evaluated were sex (male of female), age (3 or 5 days old), nutritional status (fed with sugar of blood), mortality criteria (loss of the legs) and sub-lethal effect using the CDC bottle and/or WHO modified cone methodologies. The insecticides used for assays were alfacypermethrin SC 20% and bendiocarb PM 40. Furthermore, three sand fly populations were analyzed for the designation of a reference population. The Kaplan-Meier survival curves showed a statistical difference in the sandflies diet and bioassays' methodology. Using the CDC bottle, the insects which fed on blood were more susceptible (X^2= 9,59; p = 0,00) and for WHO modified cone those insects which fed with sugar were more susceptible (X^2= 4,12; p = 0,04). Regard to age, when the population was mixed (3 and 5 days old) the lifetime was lower (240 days) than when the population was

evaluated separately (400 days). When evaluated the mortality criteria and the insecticide used (alfacypermethrin or bendiocarb), the loss of legs was observed only with the CDC bottle assay using of alfacypermethrin. Alcohol and acetone diluents did not influence the results of biological assays. The results contribute to the definition of a methodology to assess the susceptibility of *Lutzomyia longipalpis* to insecticides and allow an initial assessment of *Lutzomyia longipalpis* populations in VL endemic areas that will receive insecticide-impregnated collars.

Keywords PHLEBOTOMINES; LEISHMANIASIS; RESISTANCE; BIOLOGICAL ASSAYS; INSECTICIDE

P3-104: GEOGRAPHIC DISTRIBUTION OF *Lutzomyia whitmani* ASSOCIATED WITH VEGETATION, AND IMPACTS ON THE EXPANSION OF AMERICAN CUTANEOUS LEISHMANIASIS IN BRAZIL

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In Brazil, due to new and complex epidemiologic scenarios, the focal and dynamic transmissions of American Cutaneous Leishmaniasis (ACL) occur in different patterns, depending on location. An important example of this phenomenon is the widespread distribution and various behavior patterns of *Lutzomyia whitmani*, a vector that transmits three species of *Leishmania*: *Leishmania braziliensis*, *Leismania shawi* and *Leishmania guyanensis*. This study aims to correlate different types of Brazilian vegetation with the spatial distribution of *L. whitmani* in the areas representing Spatial Circuits of Production for American Cutaneous Leishmaniasis. In order to evaluate the ACL surveillance and monitoring model in Brazil, the Ministry of Health has analyzed the Spatial Circuit of the disease until 2013, currently adopting a classification of ACL transmission in municipalities based on the composite indicator of tegumentary leishmaniasis (ICLT). For this study, a Geographic Information System (GIS) was used to integrate the layers of *L. whitmani* geographic distribution with vegetation cover and Spatial Circuits of ACL in Brazilian municipalities. Out of the 5570 Brazilian municipalities here analyzed, information on the *L. whitmani* was found for 862. The vector occurred in nearly all types of vegetation, with a widespread distribution in: Dense Ombrophilous Forests, Open Ombrophilous Forests (or transition forests), Seasonal Decidual Forests (or deciduous woods), Seasonal

Semidecidual Forests (semideciduous woods) and Steppe. The vector was not found in Oligotrophic Woody Vegetation of the Marshes and of Sand Accumulation. This ample presence of the vector reinforces the hypothesis that *L. whitmani* is a notable species that can easily adapt to different environments. These data can be important subsidies for surveillance and prevention of ACL in Brazil by the National Leishmaniasis Control Program.

Keywords DISTRIBUTION GEOGRAPHIC; *Lutzomyia whitmani*; EXPANSION OF AMERICAN CUTANEOUS LEISHMANIASIS; VEGETATION COVER

Financing FAPERJ; INCT for Climate Change, Brazil; Brazilian Network for Research on Global Climate Change; Instituto Oswaldo Cruz/ Fiocruz

P3-105: STUDIES ON PHLEBOTOMINES (DIPTERA: PSYCHODIDAE) IN TRANSMISSION AREAS OF ATYPICAL CUTANEOUS AND VISCERAL LEISHMANIASIS IN EL SALVADOR - LATIN AMERICA

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It is estimated that leishmaniasis is responsible for approximately 2.35 million years of life lost (DALYs). The disease presents a broad clinical spectrum and visceral leishmaniasis (VL) is the most serious clinical form, causing death, when left untreated, due to the involvement of organs such as the bone marrow, spleen and liver. It mainly affects the most vulnerable people such as children under 5 years old, the elderly and patients with comorbidities and immunosuppression. According to the Pan American Health Organization/World Health Organization, LV is endemic in 13 countries in the Americas and, according to risk stratification using the triennium composite indicator of 2018-2020, El Salvador was classified as a country with low transmission of VL, however, in that same transmission cycle it presents the atypical cutaneous clinical form (ACL) caused by *Leishmania infantum (syn chagasi)*. The objective of this study was to gain knowledge on the phlebotomine fauna of El Salvador, in localities of concomitant occurrence of both clinical forms, VL and ACL, as well as to carry out the *Leishmania* sp. diagnosis and identify probable transmission areas. The captures in different areas of El Salvador were carried out in May and December 2018. The phlebotomines (males and females) were processed, clarified and assembled between slides and coverslip for taxonomic identification at the species level, according to the taxonomic key

of Young & Duncan (1994). Female samples were separated for the diagnosis of *Leishmania* sp. by molecular analysis, through the Hot-start PCR multiplex; A total of 4,538 phlebotomines of the genus *Lutzomyia* were identified: *Lutzomyia chiapanensis*, *Lutzomyia diabolica*, *Lutzomyia durani*, *Lutzomyia evansi*, *Lutzomyia gomezi*, *Lutzomyia longipalpis*, *Lutzomyia nevesi, Lutzomyia* sp., *Lutzomyia texano* and *Lutzomyia vesicifera*. The most frequent and abundant species collected in the intra and peridomicile were *Lu. evansi*, the first in rank (SISA 0.785), representing 88% of the specimens, followed by *Lu. longipalpis*, second in rank (SISA 0.452), corresponding to 8% of the processed specimens. As for sex, the largest number of phlebotomines identified were females with 66%, the same was observed with *Lu. evansi* with 70% of females, and the opposite occurred with *Lu. Longipalpis* with 83% of males. For the diagnosis of *Leishmania* sp. 242 females were sent for molecular analysis, and samples of *Lu. evansi* (one), *Lu. longipalpis* (two) and *Lu. chiapanensis* (one) were positive for *L. infantum.* Although there was no richness of the phlebotomine fauna, the findings of the two vectors, *Lu. evansi, as* the predominant species, followed by *Lu. longipalpis* involved in the transmission of *L. infantum* in the intra and peridomicile is of great importance from the epidemiological point of view for the planning and development of surveillance and control actions in the foci of transmission of ACL and VL in El Salvador.

Keywords ATYPICAL CUTANEOUS LEISHMANIASIS; VISCERAL LEISHMANIASIS; EL SALVADOR; VECTORS

Pan American Health Organization, PAHO/WHO, Oswaldo Cruz Institute, FIOCRUZ

P3-107: ANTI-SALIVA ANTIBODY PRODUCTION IN NAIVE DOGS EXPOSED TO UNINFECTED *Lutzomyia longipalpis* BITES

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Canine visceral leishmaniasis (CVL) is caused by *Leishmania infantum* and transmitted to dogs and humans by sandflies. In Brazil, *Lutzomyia longipalpis* is the primary vector of this disease. When feeding, infected sandflies inoculate metacyclic promastigote forms of *Leishmania* and their saliva and other components into the hosts. Anti-saliva antibodies were associated with increased visceral leishmaniasis severity in naturally infected dogs. Anti-sandfly saliva antibodies could also represent an essential epidemiological tool to assess vector exposure in endemic areas. LJM11 and LJM17 recombinant proteins are present in the vector's saliva and have already been used for this purpose. Our goal was to follow up anti-saliva antibodies (anti-LJM11 and anti-LJM17) production in naïve dogs experimentally exposed to *Lu. longipalpis* sandflies. We also assessed the persistence of anti-saliva antibodies titers for one year, and after re-exposure to the sandfly vectors. Blood samples from the dogs were collected weekly to assess the production of anti-LJM11 and anti-LJM17 IgG by ELISA. Six healthy naïve dogs were exposed weekly to 35 *Lu. longipalpis* female sandflies until at least 80% of the female were fed. Dogs were exposed to the sandflies until anti-saliva antibody production reached a plateau and remained elevated for at least three consecutive weeks. Afterward, we ceased sandflies exposures; we followed the dogs weekly until the animals tested negative for anti-saliva antibodies for three consecutive weeks. Then,

we re-exposed the dogs to the sandflies and evaluated the time-period it took for the animals to resume anti-saliva antibody production. The Reactivity Index (RI) was calculated by dividing the optical density by the cut-off point obtained in each ELISA plate to compare antibody production. Soon after the first exposures, there was an immediate increase in the production of anti-saliva antibodies (between the first and the third week). On the twenty-eighth day after the first exposure (with a median of 10.5 days), all six animals showed detectable anti-saliva IgG titers. Dogs were exposed to sandflies for six to nine weeks (with a median of 52.5 days). After the initial rising of anti-saliva antibody production post-exposure, anti-saliva antibody titers fluctuated, remaining detectable for over a year. We found a statistically significant difference comparing anti-saliva antibodies titers before exposure and five weeks after the exposure ($p<0,05$). Despite the variations in titration, four dogs remained positive for 41 weeks (290 days) on average, two animals are still positive after 460 days. After the first week of re-exposure, dogs that were re-exposed demonstrated antibody titers rising significantly. Throughout the evaluation, there was a considerable variation in antibody production among the six animals, especially concerning the time of seroconversion, time to reach the plateau, and titer decay. Although we observed differences among the animals, we can detect a similar pattern during the follow-up. Currently, studies evaluating the cellular immune response of these animals are being carried out. Assessing anti-sandfly saliva antibodies can help understand vector exposure dynamics in endemic areas, using a relative non-expensive tool.

Keywords SANDFLY; SALIVA; ANTIBODIES; RESERVOIR

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P3-108: MORPHOLOGICAL DIVERSITY OF SALIVARY GLANDS OF *Phlebotomus argentipes*

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Sand fly saliva contains powerful pharmacologically active substances. Yet very little is known about the characteristics of salivary proteins. Number and amounts of salivary proteins produced in salivary glands correlate with the size of the glands and vary with diet and age of sand flies. Therefore, optimal age of sand flies to be used in dissections to obtain salivary glands is critically important for experiments related to sand fly salivary proteins. The present study gives the morphological details and dimensions of salivary glands of *Phlebotomus argentipes*, the probable vector of cutaneous leishmaniasis in Sri Lanka to help in determining the optimal age of sand flies to perform the salivary gland dissections. *Ph. argentipes* sand flies were maintained in the insectary and 1 to 10 days old female sand flies (8 flies for each day) maintained on 30% sucrose feed and 7 days old blood-fed flies and were anesthetized at –20ºC for 10 minutes and dissected over a glass slide in cold phosphate-buffered saline (PBS), pH 7.2. The sand fly head was slowly detached from the thorax until it is completely separated from the body. Width and length of salivary gland lobules were measured with an inverted microscope with objective lenses calibrated with a micrometer. Salivary glands of female *Ph. argentipes* are paired, transparent, hollow organs surrounded by an outer epithelium and heterogeneous in terms of size and shape. Small droplets like epithelial secretory cells were observed in the glands of sugar-fed flies and number of these cells increased with age but secretory cells were not seen in 7 days old blood-fed flies. Mean length and width of major lobe of one day old female flies were 269.8 ± 13.0 µm and 198.5 ± 17.2 µm respectively and minor lobe was 204.0 ± 13.6 µm and 173.0 ± 18.6 µm. Mean lengths and widths of both lobes of salivary glands significantly increased ($p < 0.05$) from first day to seventh day. However,

increment rate of the sizes of the salivary gland lobes plateaued after 7 days while survival rate of flies gradually reduced after 8 days (1/8 died, 12.5%). The mortality percentage was 50% (4/8) at 10 days. Significant size difference ($p < 0.05$) was observed between 7 days sugar-fed and blood-fed flies where the mean lengths (major lobe: 279.1 ± 17.2 µm, minor lobe: 218.3 ± 15.5 µm) and widths (major lobe: 254.2 ± 20.8 µm, minor lobe: 198.0 ± 6.6 µm) of both lobes of 7 day old blood fed flies were less than that of 7 days old sugar fed flies (lengths; major lobe: 365.6 ± 12.2 µm, minor lobe: 305.1 ±19.8 µm; widths: major lobe: 305.1 ± 15.8 µm, minor lobe: 252.3 ± 11.3 µm). The size differences of salivary glands varied with the age and diet of female sand flies. Based on the study observations, the optimal age range for dissection of sand fly salivary glands was 5 to 7 days. Sugar fed sand flies are better for experiments on salivary glands.

Keywords SALIVARY GLANDS, SALIVARY PROTEINS, *Phlebotomus argentipes*, MORPHOLOGY, VECTOR

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P3-109: THE DETECTION OF NATURAL INFECTION IN *Trichophoromyia viannamartinsi* BY *Leishmania braziliensis* IN AN AREA OF AMERICAN TEGUMENTARY LEISHMANIASIS IN SOUTHEASTERN BAHIA, BRAZIL

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This ongoing descriptive study aims to characterize of the phlebotomine fauna found around and near residences of newly diagnosed American tegumentary leishmaniasis (ATL) cases in the Cacao Region at southeastern Bahia, Brazil. This region is highly endemic for *Leishmania braziliensis*. The local sand fly fauna was studied from may/2018 to july/2019 by Entomological Survey, as recommended by the Brazilian Ministry of Health. In this period, 619 sandflies of 20 species were captured: 273 males (44%) and 346 females (56%). *Nyssomyia whitmani* was the most prevalent (62.2%), followed by *Nyssomyia intermedia* (9.2%), *Evandromyia bahiensis* (6.3%), endemic in Bahia, and *Th. viannamartinsi* (4.5%). Ninety-four percent of the collected females were screened for natural infection with *L. braziliensis* by Polymerase Chain Reaction. Of 97 sand fly *pools* analyzed, seven were positive for *L. braziliensis*: three of *Ny. whitmani*, two of *Th. viannamartinsi* and one each of *Psychodopygus davisi* and *Trichopygomyia longispina*. The minimum infection rate (MIR) found was 2.2% and, for the mentioned species, 1.94%, 10%, 33% and 50%, respectively. The PCR was sensitive to detect infection even in samples with only one individual of the species *Th. viannamartinsi* and *Ty. longispina*. All positive *pools* came from CDC traps installed in peridomiciles and extradomiciles of homes during july/2018 in the municipality of Taperoá. Individuals collected in this incursion displayed MIR of 7.8%, with 50% of the *pools* positive for *L.*

braziliensis. To our knowledge, *Th. viannamartinsi* natural infection by *L. braziliensis* had not yet been reported in the literature. This species was described in the 1970s, and was initially considered in synonymy with *Trichophoromyia brachipyga*. *Trichophoromyia* spp. has not been extensively studied, but its epidemiological relevance is suggestive in Amazonian states, where it is abundant in urban areas endemic for ATL and has been found naturally infected with *L. braziliensis*. The results found in this research consist on the initial step in our attempt to better characterize the ecoepidemiological cycle of *L. braziliensis* in southern Bahia. There is a *Modified Silvatic* pattern in a forest vegetation region, where enzooty occurs in wild mammals, with the participation of exophilic species as *Ps. davisi*, *Th. viannamartinsi* and *Ty. longispina*. The peridomiciliary transmission involves the parasite circulation in domestic and synanthropic mammals that can serve as a source of infection manly for endophilic species *Ny. intermedia* and *Ny. whitmani* and as well maybe *Migonemyia migonei* and *Pintomyia fischeri* that consequently infect humans. We suggest increasing focus on the study of human-biting behavior, vector competence and epidemiological relevance of *Th. viannamartinsi* in ATL endemic areas at the bahian Cacao Region, in Northeast Brazil.

Keywords CUTANEOUS-LEISHMANIASIS; PHLEBOTOMINAE; VECTOR; BAHIA; BRAZIL

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P3-110: COMPARISON OF TWO SANDFLIES CAPTURE METHODS IN URBAN AREA FOR MONITORING *Lutzomyia longipalpis* POPULATIONS

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Lutzomyia longipalpis, the main vector of visceral leishmaniasis agent, is an urbanized sandfly in Brazil, and monitoring its distribution is essential for the control initiatives. Two methods are recommended for the entomological surveillance of sandflies, CDC light traps and manual captures using electric aspirators. However, the efficiency of these methods has been poorly evaluated. For the implementation of an entomological surveillance system on a large scale in a municipality this evaluation is essential considering the scarcity of human resources and technical capacities. Therefore, this study evaluated the efficiency of two sandflies capture methods and the distribution of *Lu. longipalpis* to identify entomological indicators for addressing Entomological surveillance activities in the VL control program. All households in urban area of the municipality were evaluated for peridomicile characteristics (presence of peridomicile, vegetation or organic material, dogs, henhouse, other animals shelters). For each feature present, a point was assigned. A vector risk score was defined. Then 466 households were sampled for sandflies captures with a manual aspirator and 140 with the higher scores were selected to undertake captures with CDC light traps. The captures were undertaken between October 2020 to May 2021. A total of 2347 specimens of *Lu. longipalpis* were captured (1944 males and 528 females), 43.1% in the light traps and 56.9 in the manual aspirator. Among 271 females captured in the light traps, 29 were engorged (10.7%) while in the manual capture were 51.5% out of 132. Of the total *Lu. longipalpis* specimens 33% and 9% were captured in

domiciles with chicken houses and kennels, respectively, suggesting these environmental characteristics as the main indicators for sandflies occurrence. As regards the comparison of the capture methods, the manual aspirator was more efficient. With this method, 466 households were screened detecting sandflies in 22% of them (9 h45min on average, 20 minutes/per domicile). With CDC light traps, among 140 households 75% were positive, the capture effort was higher, although (420 capture attempts), in total 6478 hours of exposure. The male: female ratio was 10:1 in CDC light traps and 2.5:1 in manual aspirators, as well as the number of engorged females was higher in the last one, representing 70% of the total engorged females. However, it is important to remark that these light traps are operationally advantageous, although the short time (20 min) manual aspirator was well accepted by the residents of the domiciles. Monitoring of vector population is the basis of a control program for developing entomological surveillance activities. In this study, we observed that CDC light traps show a high efficiency to detect the presence of *Lu. longipalpis* in an urban area and offer the possibility of sampling large areas. However, the manual aspirator seems to be more efficient when the goal is to identify blood meal sources. Both methods are well accepted by the residents of domiciles and provide key information for the management VL program.

Keywords *Lutzomyia longipalpis*; ENTOMOLOGICAL SURVEILLANCE; CDC LIGHT TRAP; MANUAL ASPIRATOR; EFFICIENCY

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P4-065: SANDFLIES DIVERSITY AND TEMPORAL DISTRIBUTION IN AN ENDEMIC AREA OF CUTANEOUS LEISHMANIASIS OF CALDAS DEPARTMENT-COLOMBIA.

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Sandfly species diversity in Colombia is high, 172 species, 14 of them are suspected or proven vectors of *Leishmania* sp. Colombia present a wider range of altitude and variability in its geographic features, that can affect the ecological structure of the sand flies, influencing parameters such as richness, abundance and temporal distribution. Temperature and rainfall influence the life cycle duration, the parasite extrinsic incubation and the gonotrophic cycle, key factors related to the vector capacity. Understanding the effect of changes in meteorological variable on vector dynamics at local scale is important to predict the vector distribution and for planning disease management and control strategies. The municipality of Victoria (Caldas, Colombia) is endemic for cutaneous leishmaniasis, where cases are reported each year, occurring mainly in the rural area. Although the presence of species vector is known, the population dynamics and how they could be affected mainly in the rainy season is unknown. This study aims to describe the temporal distribution of sandflies and the association with the rainy season and other meteorological factors. This study was conducted from November 2020 to October 2021. Sandfly samples were obtained monthly with CDC light traps installed in the peri and intradomicile of six residences, for three consecutive nights. Data about temperature, relative humidity and rainfall were obtained throughout the period by using a portable weather station. The sand fly species identification was performed using the Galati key. 4,602 sandflies were collected, distributed in 20 species and 11 genera. *Nyssomyia yuilli yuilli, Psychodopygus ayrozai* and *Ps. panamensis*, known

anthropophilic species, predominated throughout the period, representing 83.1% of the total specimens. In all the months of the year, the most abundant species was *Ny. yuilli yuilli,* the other species varied slightly in abundance. In November, the month just before the increase in rainfall, occurred the highest abundance and richness of sandflies. The species *Evandromyia dubitans, Ev saulensis, Psathyromyia barrettoi majuscula* are reported for the first time for the Caldas department. In the ecological context, the new records of species for the department support the importance of entomological surveys to know the ecological community of sandflies in this region. In the epidemiological context, the capture of sandflies during all year in the domicile and the predominance of anthropophilic species in the study area suggest a constant exposition of the human population with vector and agents of leishmaniasis and an increase odd of leishmaniasis cases occurrence.

Keywords SANDFLY; DISTRIBUTION; METEOROLOGICAL FACTORS

P4-067: *Leishmania* IN SANDFLIES FROM A LEISHMANIASIS MIXED FOCUS IN A RURAL AREA OF OVEJAS, NORTHERN COLOMBIA.

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Leishmaniasis are a group of diseases of parasitic origin that affects the skin (Cutaneous Leishmaniasis, CL), mucous membranes (Mucocutaneous Leishmaniasis, MCL), and internal organs (Visceral Leishmaniasis, VL). The study area was El Palmar, rural area of the Ovejas municipality (Sucre department)- This municipality belong to the Colombian Caribbean region and is considered as one of the three most important areas in the country for VL. In this locality the known and proved vector for VL is *Lutzomyia evansi* although vectors for CL remains unknown in spite of the growing incidence of this form of the disease in several areas of this municipality. Entomological sampling was carried out using CDC light traps between 18:00 to 06:00 at the intra, peri and extradomicile of houses of three CL patients. Female sandflies were morphologically identified by taxonomic keys of Young & Duran (1994) and supported by descriptions of Galati (2019). Unfed females from the same sandfly species and house environment (intra, peri and extradomicile) were pooled together (until 10 females by pool), whereas blood-fed females were treated individually. Sandflies were submitted to DNA extraction and tested for ribosomal ITS1 region of *Leishmania*. Blood-fed females were also tested for human DNA bloodmeal using a highly specific Cytochrome B PCR. Male phlebotomine sandflies included 977 insects from 8 sandfly species: *Lu. evansi* (903 specimens), *Lutzomyia gomezi* (36), *Lutzomyia panamensis* (17), *Lutzomyia c. cayennensis* (10), *Lutzomyia dubitans* (4), *Lutzomyia trinidadensis* (3), *Lutzomyia rangeliana* (2) and *Lutzomyia carpenteri* (2). On the other hand, 577 sandfly females were collected and tested, including 311 female blood-fed individuals and 266 unfed females (as pooled insects). Collected female

sandflies were identified as *Lu. evansi* (541), *Lu. gomezi* (11), *Lu. panamensis* (11), *Lu. c. cayennensis* (11 specimens) and *Lu. dubitans* (3). *Leishmania* DNA was detected in 21 individually analyzed blood-fed females: 18 *Lu. evansi* (4 intra, 14 peridomicile), 1 *Lu. gomezi* (peri) and 2 *Lu. panamensis* species (intra and peri). None of the blood-fed phlebotomines were positive for human DNA, and none of the 266 unfed insects tested by pools were positive to *Leishmania* DNA. DNA sequencing and phylogenetic of PCR amplicons will be the next step to identify the parasites species and help to clarify the transmission cycles of this complex disease in this region of South America.

Keywords VECTORS; *Leishmania*; BLOODMEAL; PCR; CUTANEOUS LEISHMANIASIS

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P4-068: FIRST REPORT OF PHLEBOVIRUSES CIRCULATION IN PHLEBOTOMINE SANDFLIES COMMUNITIES FROM NORTHERN COLOMBIA.

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Phlebotomine sandflies are mainly recognized as vectors of *Leishmania* parasites in the Americas, however, they are also potential vectors of some other parasites, bacteria, and finally, some viruses. Among the main viruses associated with sandflies, the *Phlebovirus* genus (Bunyavirales: Phenuiviridae) stands out as the etiological agents of different human diseases in the Old and New World. In Colombia, the knowledge about phleboviruses is limited to the base work developed by Tesh and collaborators, although it is possible the existence of silent transmission cycles of those viruses in this country given the anthropophilic sandfly fauna and the ecological conditions. The main goal of this work was to monitor the silent circulation of phleboviruses among the sandflies communities in northern Colombia. The entomological survey was performed in five municipalities from the Caribbean region of Colombia: Toluviejo, Purísima, Sincelejo, San Juan Nepomuceno and Montería. Sandflies were collected through CDC (18:00-06:00 hrs) and Shannon (18:00-22:00 hrs) traps. Alive sandflies from each area were pooled together with up to 140 individuals per pool. Additionally, specimens of different morphotypes were selected for taxonomic identification from each locality. The sandfly pools were used for RNA extraction, retrotranscription, and a nested-PCR for amplification of the RdRp gene located in the L segment of the phlebovirus genome. A total of 5955 specimens organized in 58 pools, wich were used in molecular assays and four of them tested positive for phleboviruses presence. The phylogenetic analysis of the

sequences let us identify a strain of the Armero virus (Aguacate serocomplex; aminoacidic p-distance <5%), a strain of Capira virus (Punta Toro complex; aminoacidic p-distance <5%), and a Sloths-related phlebovirus (aminoacidic p-distance >5%) in the Toluviejo municipality, where It is important to highlight the presence of highly anthropophilic sandflies such as *Lutzomyia evansi*, *Lutzomyia panamensis* and *Lutzomyia olmeca bicolor*. A Phlebovirus related to the pathogenic Old World group (aminoacidic p-distance >5%) was detected in Sincelejo (Sucre department), where *Lutzomyia evansi* and *Lutzomyia panamensis* were morphologically identified. Two of the detected phleboviruses are probably new viral species given the aminoacidic distance defined as a threshold by ICTV and grouping pattern in the phylogenetic tree. The results here presented are the first report of Phlebovirus genus in the Caribbean re-gion from Colombia, and it becomes more relevant since the areas where viruses circula-tion is confirmed as areas of notification for leishmaniasis cases, where anthropophilic sandflies species are involved. Furthermore, the silent transmission cycles can be over-looked because of the diagnosis of common viral febrile syndromes and *Leishmania* infection itself, therefore, more studies concerning to those agents need to be carried out to determine the vector sandfly species and their impact on human populations.

Keywords SANDFLIES; PHLEBOVIRUS; COLOMBIA; CARIBBEAN; PUNTA TORO COMPLEX

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P4-070: BIOACTIVITIES OF INTESTINAL BACTERIA OF TROPICAL DISEASE VECTORS

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A better understanding of vector-microbiota-pathogen interactions is vital, as it can lead to the discovery of new tools to block disease transmission and provide critical information for the development of intervention strategies for sand fly control or mosquitoes as *Aedes.* Data on the microbial consortium associated with sand fly and mosquitoes microbiota have highlighted that microorganisms influence many aspects of host biology including their survival, development, and vector competence. In this study, enzymatic, antimicrobial and larvicide bioactivities were evaluated to determine the biotechnological potential of bacteria isolated from the intestines of *Pintomyia evansi* and *Aedes aegypti.* The identity of the bacterial isolates was confirmed using two molecular markers (16S ribosomal DNA gene and gyrβ gene). The enzymatic activities were evaluated in Petri dishes with mineral media and three different carbon sources (starch, blood and skim milk). Larvicide activity was evaluated against early stage L4 larvae of *Aedes albopictus.* Finally, the antimicrobial activity was tested against Gram positive and Gram negative reference bacteria. These last two activities (Larvicide-Antimicrobial) were developed using crude methanolic extracts. The bacteria that showed hemolytic activity were *Bacillus anthracis*, *Bacillus safensis* and *Chryseobacterium* sp; which together with *Rummelibacillus stabekisii* and *Priestia (Bacillus) megaterium* showed proteolytic activity. Amylase activity evaluated with starch degradation and revealed with lugol, was positive for *Ochrobactrum anthropi*, *Bacillus megaterium* and *Bacillus anthracis*. On the other hand, the larvicide activity of extracts against L4 larvae of *Aedes albopictus* presented high percentages of mortality (70-

100%) for *Bacillus safensis* and *Chryseobacterium* sp mainly. It is necessary to specify that the crude extract of *B. safensis* presented an antagonistic activity against *S. aureus* and *B. cereus.* Additionally, the *Elizabethkingia anophelis* strain showed activity against *B. cereus.* It is necessary to characterize the metabolites involved in the different bioactivities to better specify or enhance the targets and mechanisms of action against vectors or pathogens.

Keywords MICROBIOTA; BIOACTIVITIES; *Aedes;* LEISHMANIASIS*; Pintomyia evansi;* BIOCONTROL

P4-071: EPIDEMIOLOGICAL STUDY OF PHLEBOTOMINAE FAUNA (DIPTERA: PSYCHODIDAE) IN TINGUÁ, MUNICIPALITY OF NOVA IGUAÇU, STATE OF RIO DE JANEIRO, BRAZIL

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American Cutaneous Leishmaniasis (ACL), classically considered a zoonosis of wild animals, has outbreaks related to environmental factors, which can cause changes in the epidemiological profile of the disease. Forest parks open to the public can offer favorable conditions for the maintenance of *Leishmania* spp cycles and, consequently, be a potential risk of ACL infection. Thus, the objective of the study was to contribute to the epidemiological knowledge (fauna study, diagnosis of Leishmania spp. and assessment of food content) of sandflies in the Tinguá Federal Biological Reserve - REBIO and in the Tinguá Environmental Protection Area - APA, municipality of Nova Iguaçu, Rio de Janeiro. The captures were carried out from September/2019 to March/2020. The captured sandflies (male and female) were processed, clarified and mounted for taxonomic identification at species level. Samples from females were separated for the diagnosis of *Leishmania* sp. and food content, by molecular analysis, through multiplex Hot-start PCR and Sequencing. A total of 2,172 sandflies were identified, belonging to eight genera and 16 species, *Brumptomyia brumpti*, *Brumptomyia cardosoi*, *Brumptomyia nitzulescui*, *Evandromyia edwardsi*, *Evandromyia termitophila*, *Micropygomyia quinquefer*, *Migonemyia migonei*, *Nyssomyia intermedia*, *Psathyromyia pascalei*, *Psathyromyia pelloni*, *Psathyromyia lanei*, *Pintomyia fischeri*, *Pintomyia misionensis*,

Psychodopygus ayrozai, *Psychodopygus davisi* and *Psychodopygus hirsutus hirsutus*. The most abundant species captured in REBIO and APA were, *Ps. hirsutus hirsutus* (SISA 0.41), corresponding to 39% of the specimens, followed by *Ps. davisi* (SISA 0.37), representing 43%, and *Ny. intermedia* (SISA 0.18), with 9% of the specimens processed. Being *Ps. hirsutus hirsutus* more abundant in REBIO, and *Ps. hirsutus hirsutus* and *Ny. intermedia* in the APA. Regarding gender, females corresponded to 51% of the specimens. For molecular diagnosis, of the 213 females analyzed, a sample of *Ps. hirsutus hirsutus*, was positive for *Leishmania (Viannia) braziliensis*, and a female of *Ps. davisi* was detected fed on blood of *Tamandua tetradactyla*. The results on the sandfly fauna allowed us to identify, for the first time in Tinguá, species of medical importance, possibly involved in the transmission of *Le. (Viannia) braziliensis* in the state of Rio de Janeiro, *Ny. intermedia*, *Mg. migonei*, *Pi. fischeri* and *Ps. hirsutus hirsutus*, in addition to the *Ps ayrozai* record, which was found to be infected by *Leishmania (Viannia) naiffi*. It is worth mentioning that the municipality of Nova Iguaçu is considered endemic for ACL, where *Ny. intermedia* is the predominant species in periurban areas. In this pioneering study, the species *Ny. intermedia* more abundant in a residential area, close to a forest with intense anthropic alteration and presence of domestic animals, where a human case of ACL has already been reported. Species of the *Psychodopygus* subgenus showed greater abundance in the forest, and were also captured at home in more preserved areas. The positive diagnosis of *Le. (Viannia) braziliensis* in a female of *Ps. hirsutus hirsutus*, may be suggestive of the possible occurrence of a sylvatic cycle in the REBIO area.

Keywords NOVA IGUAÇU; LEISHMANIASIS; PHLEBOTOMINAE; REBIO

P4-072: EXPERIMENTAL EVALUATION UNDER LABORATORY CONDITIONS OF INSECTICIDAL PAINT AGAINST COLOMBIAN *Rhodnius prolixus* AND *Triatoma dimidiata* (HEMIPTERA: REDUVIIDAE)

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Rhodnius prolixus and *Triatoma dimidiata,* are the main vectors of the parasite *Trypanosoma cruzi,* the causative agent of Chagas' disease, in Colombia. Under the Andean Countries Initiative, the National Health Ministry of Colombia implemented a program to eliminate the domestic vector *R. prolixus* in order to interrupt the transmission of *T. cruzi*. Traditionally, spraying techniques are used for the elimination of domestic *R. prolixus* in prioritized areas. However, *T. dimidiata* became the main vector in areas once infested by *R. prolixus*. The large-scale insecticide application is costly and need numerous trained personal. For this reason, brand new alternative control techniques are vitally required. We evaluated the residual effect of insecticidal paint on the mortality of five instar nymphs of *R. prolixus* and *T. dimidiata.* The study experimental design included two groups treated with paints containing the pyrethroids-based Insectex®. Adobe bricks were prepared by applying either commercial water-based paint or oil-based paint. Three evaluations were made: time up, three and six months after application of paint. Here we evaluated the mortality of 40 nymphs 24 and 48 hours after exposure to the painted blocks: 10 nymphs per treatment, as three repetitions and an untreated control. Insectex® showed long residual activity, causing 100% mortality of five instar nymphs of both *R. prolixus* and *T. dimidiata* after both three and six months after paint application. No mortality effect was registered in the untreated controls after 48 hours. We demonstrate that the pyrethroids-based Insectex®evidenced a long residual activity on the mortality of five instar nymphs of *R. prolixus* and *T. dimidiata* under laboratory conditions. The application of this paint along with housing improvement could be

considered as an alternative control tool to spraying in areas with both vector infestation. This method has the potential to interrupt the transmission of *T. cruzi* by this domestic vector in Colombia.

P4-073: GUT MICROBIOTA OF *Lutzomyia longipalpis* FROM RICAURTE (CUNDINAMARCA) EXPERIMENTALLY INFECTED WITH *Leishmania infantum* AND *Leishmania braziliensis,* REVEALS HIGH DOMINANCE OF *Pseudomonas* AND ENTEROBACTERIACEAE

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The sand fly gut microbiota has recently emerged as an encouraging field to be explored for vector-based disease control. Previous studies reported that variation in the microbiota residing in the insect gut might be mainly explained by the effects of host habitat, diet, developmental stage, and phylogeny, all contributing to the structure of insect gut microbiota. However, a better understanding of vector-microbiota-pathogen interactions is vital, as it can lead to the discovery of new tools to block disease transmission. Diverse data suggest that the sand fly midgut microbiome is a critical factor for *Leishmania* growth and differentiation to its infective state prior to disease transmission. This study aimed to defining the structure and diversity of the gut microbiota of *Lu. longipalpis* in response to *Leishmania infantum* and *Leishmania braziliensis* infection under laboratory conditions. Entomological sampling was performed in 2021, during a period of high rainfall, in the Callejon locality, in the Municipality of Ricaurte. For this, the females were captured using a buccal aspirator on resting sites and Shannon trap. To evaluate the effect of the gut microbiota of *Lu. longipalpis* on its susceptibility to infection with *Leishmania,* several groups were considered: blood-fed females with *L.*

infantum; blood-fed females exposed to *L. braziliensis*; blood-fed females (control); and a group of females naturally infected with *L. infantum*. *Leishmania infection* was diagnosed by visualization of parasites in the digestive tract (6-7 days post-infection*)* and PCR (KDN3 Marker). DNA was obtained from several groups of guts of *L. longipalpis* using the ZR Tissues & Insect DNA miniPrep, and PCR amplicon libraries of the 16S rDNA V4 region were prepared using total DNA as a template. The PCR products were subjected to 250 bp paired-end Illumina MiSeq sequencing. Taxonomy and diversity metrics were estimated using the phyloseq software package and Microbiome Analyst. The midgut microbial population of *Lu. longipalpis* was composed mainly of Proteobacteria (90%), followed by Firmicutes (5%), Actinobacteria (2%), and others (3%). In blood-fed females and the group exposed to *L. infantum, however uninfected, Pseudomonas* has a high relative abundance (75–85%), in contrast to guts positives to the infection for *L. infantum* (30–55%) under experimental infection or gut of females naturally infected with the same species of the parasite (35%). ASVs that moderately increased in guts infected with *L. infantum* were Enterobacteriaceae (25%), *Enterobacter* (10%), and *Klebsiella* (5%). The group positive for *L. braziliensis* under experimental infection, showed a higher relative abundance of *Pseudomonas* (95%) and in less proportion of *Enterobacteriaceae* regarding the guts with *L. infantum.* The females naturally infected with *L. infantum* showed the highest ASV diversity. Also, some endosymbionts such as *Rickettsia* (in females negatives for *L. infantum*), *Cardinium* (in females naturally infected), and *Spiroplasma* (blood-fed females) were detected in the DNA of the gut of females of *Lu. longipalpis* with low abundance. This study showed that the presence of *L. infantum* or *L. braziliensis* can reduce the richness and diversity of microbiota and that a differential abundance of *Pseudomonas* can be recorded according to the species of *Leishmania*. Finally, in vivo co-infection studies are needed to better understand *Leishmania*–microbiota–sand fly interactions.

P4-074: EVALUATION OF THE DEVELOPMENT OF *Leishmania (Viannia) braziliensis* AND *Leishmania (Leishmania) infantum* ON *Migonemyia migonei* AND *Lutzomyia longipalpis*

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Leishmaniases are important neglected diseases and *Leishmania (Leishmania) infantum* and *Leishmania (Viannia) braziliensis* are the most important causative agents of leishmaniasis in the Americas. These two species of parasites can co-circulate in a specific endemic area, but their interactions with no vectors have not yet been studied. We evaluated experimental changes using simple infections and co-infections to compare the development of *L. (L.) infantum* (OGVL / mCherry) and *L. (V.) braziliensis* (XB29 / GFP) in *Migonemyia migonei* and *Lutzomyia longipalpis*. The level of infection and the location of the parasites were analyzed in females dissected on the 2nd, 5th and 8th days after blood meal, using fluorescence microscopy. Both *Leishmania* species completed their life cycle, producing infective forms in both species studied. Analyzing the co-infection, based on the difference in fluorescence, all females of *Mg. migonei* were infected with both species, and the parasites were confined to the peritrophic matrix. It was observed that the level of infection was high on the 2nd day for both species. On the 5th day, it was possible to observe a high level of infection for *L.* (*L.*) *infantum*, both in coinfections and in simple infections, and in 20% of females, the parasites colonized the stomodeu valve. The rate of infection

by *L.* (*V.*) *braziliensis* was lower, 56% for co-infections and 50% for simple infections. On the 8th day, in coinfections *L.* (*L.*) *infantum* overlapped *L.* (*V.*) *braziliensis* and colonized the stomodeal valve in large numbers, and in simple infections we observed 90% of females infected with *L.* (*L.*) *infantum*, 68% infected with *L.* (*V.*) *braziliensis.* In *Lu. longipalpis*, it was possible to observe that on the 2nd day the infection rate was high in all parasite-vector combinations. On the 5th day the coinfection was 91% and 54% for *L.* (*L.*) *infantum* and *L.* (*V.*) *braziliensis*, in simple infections the infection by *L.* (*L.*) *infantum* remained high and there was a decline in the infection by *L.* (*V.*) *braziliensis*. On the 8th day of infection, were observed 75% with *L. (L.) infantum* and 66% of the samples infected with *L.* (*V.*) *braziliensis*, the same was observed in the coinfection. The same occurs in coinfections, demonstrating that the two parasites complete their development and do not compete with each other. Thus, infections produced by *L. (L.) infantum* reached higher rates and grew faster than *L. (V.) braziliensis*. In the 5th and 8th day post-infection changes, *L. (L.) infantum* was present in all regions of the midgut, such a typical suprapilarian development, while *L. (V.) braziliensis* was concentrated in the hindgut and abdominal (peripillar development). From these results it is possible to conclude that the two species of sandflies are permissive to the concomitant development of the two parasites and more susceptible to the development of *L. (L.) infantum*. An unprecedented and important finding for public health, contributing to a better understanding of the transmission cycle of leishmaniasis and the parasite-vector interaction.

Keywords LEISHMANIASIS; *Leishmania*; CO-INFECTION; *MIgonemyia migonei; Lutzomyia longipalpis*

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P4-075: EXPERIMENTAL INFECTIONS OF RODENTS WITH *Leishmania* (*Mundinia*)

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Mundinia is a recently described subgenus of *Leishmania* (former *L. enrietti* complex). Several species belonging to this subgenus infect humans but their reservoir hosts are unknown. Experimental models for laboratory research play an important role in understanding natural cycles, parasite pathogenicity and host immune responses to leishmania parasites. Laboratory mice, golden hamsters and dogs were established as experimental models for research on *Leishmania* and *Viannia* subgenera but a reliable laboratory model for most *Mundinia* species is missing. Here, Balb/c mice, Chinese hamsters (*Cricetulus griseus*), and Steppe lemmings (*Lagurus lagurus*) were infected intradermally with three human infective *Mundinia* species: *L. martiniquensis*, *L. orientalis*, and *L.* sp. from Ghana. Xenodiagnoses were performed every 5 weeks post infection (p.i.) and the experiment was terminated at week 20 p.i. by dissection of the animals. All collected tissues were stored for qPCR analysis. While Balb/c mice and Chinese hamsters did not show any external signs of infection during the experiment, Steppe lemmings proved susceptible to all three species tested. Skin lesions were observed in Steppe lemmings infected by *L. martiniquensis* and *L.* sp. from Ghana, often leading to complete destruction of the inoculated ear. More severe signs of visceral leishmaniasis, such as hair loss, weight decrease and cachexia, were observed in animals infected with *L. orientalis*, leading to 40% mortality. Analysis of tissue samples is still ongoing, but the results obtained so far support the finding that Steppe

lemmings can serve as a good experimental model for research on *Mundinia* parasites.

Keywords *Leishmania*; *Mundinia*; EXPERIMENTAL MODEL; STEPPE LEMMINGS; RODENTS

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P4-076: *Leishmania infantum* INFECTION IN STRAY CATS IN AN ENDEMIC AREA FROM BRAZIL DURING COVID-19 PANDEMIC.

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Zoonotic leishmaniasis caused by *Leishmania infantum* is a disease that requires attention, especially in endemic areas, where environmental factors, human and animal cases are interconnected. Knowledge of mammal infections in endemic areas is crucial for developing control strategies. Recent investigations have focused on domestic cats are potential hosts and may acquire *L. infantum.* The presence of *L. infantum* infection is associated with altered immunocompetence based on immune depletion, predominant T-helper 2 (Th2) and T-helper 1 (Th1) responses. Studies carried out suggest that immunosuppressed animals might be especially susceptible to SARS-CoV-2 infection. This work aimed to investigate *L. infantum* infection's in stray cats from an Urban Park in Brazil during the COVID-19 pandemic. To date, there have been no previously reported cases of feline leishmaniasis (FeL) in this studied area. From February to September 2021, at Parque Municipal Américo Renné Giannetti, Belo Horizonte, Minas Gerais, Brazil, 80 stray cats of mixed breeds were tested for *L. infantum*. To identify *Leishmania* ssp. ear DNA and digestion of the ITS1 amplicon with the restriction enzyme *Hae*III (RFLP-PCR) samples, were used. The PCR-RFLP products were compared with *Leishmania* spp. samples. ITS1-PCR products were purified and sequenced. The sequence analysis was performed using GenBank. *L. infantum* infection was confirmed by PCR-RFLP and sequencing in 15% (12/80) of cats; of these 8,75% (7/80) were females and 6,25% (5/80) were males. No animal in this study exhibited at least one clinical

sign of Leishmaniasis. In the cat population, 91,25% (73/80) were adults, 1,25% (1/80) young and 7,5% (6/80) kitten cats, of these 53,75 (43/80) were females and 46,25% (37/80) males. Cats are susceptible to *Leishmania* infection and the possibility of SARS-CoV-2 contamination could aggravate existing health conditions and lead to a decreased immune response, leading to rapid disease progression. The Covid-19 pandemic has been causing concern across the world, and both, animal adoption and abandonment have increased during these times. The geographic area analyzed is an important tourist site, frequented daily by people, domestic and sylvatic animals. The detection of *L. infantum* in stray cats that walk through the park raises concern, as it is not known the impact of transmission of the parasite associated with the severe acute respiratory syndrome Coronavirus 2, which can also affect these animals. This is the first study reporting *L. infantum* infection in stray cats at Parque Municipal Américo Renné Giannetti, Belo Horizonte, Minas Gerais, Brazil. Molecular identification and sequencing of *L. infantum* in these animals indicate that the parasite is circulating in ZVL endemic areas. All this reinforces the importance of monitoring zoonotic diseases, which may transmit from animals to humans. The need for surveillance is recommended to clarify the importance of felines in the ZVL cycle that may be carriers of SARS-CoV-2.

Keywords *Leishmania infantum;* SARS-COV-2; CAT; PCR-RFLP; LEISHMANIASIS

Financing CAPES, FAPEMIG.

P4-078: *Ctenodactylus gundi* AS RESERVOIR HOST OF *Leishmania* PARASITES IN TUNISIA

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Leishmaniasis is a public health problem since it shows a real geographic extension and affects both humans and animal species. Several rodent species have been associated with the disease transmission cycle. However, it would be necessary to suspect other rodents living in endemic areas, since there are foci of CL where the main reservoirs are unknown. *Ctenodactylus* (*C.*) *gundi* has been suspected to be the reservoir of *Leishmania* (*L.*) *killicki* for some decades in Tunisia. The objective of this present work is to estimate the prevalence of leishmaniasis infection among *C. gundi* using parasitological and molecular tools and to predict its role in the transmission of this pathology. An eco-epidemiological investigation was carried out in the south-east of the country for the capture of this wild rodent in these endemic areas. Between 2016 and 2017, a number of 240 C. gundi were captured in the south-eastern part of Tunisia. Direct examination, using macerated ears, identified 72 (30.8%) *C. gundi* with leishmaniasis. The ITS1-PCR technique of spleen biopsies demonstrated the presence of *Leishmania* DNA in 148 rodents with a rate of 61.66%. The study

of the infection prevalence according to the season of capture showed that these rodents are exposed throughout the year, it worsens in Autumn and decreases significantly in Summer. In addition, no significant difference by sex of the rodents was determined. RFLP analysis of ITS1-PCR products revealed that 80 samples were identified as *L. major* with an infection percentage of 54.1% which ranged from 3.8% to 41.3% in Summer and Autumn, respectively. Although 38 were identified as *L. killicki* (25.7%) with an infection rate ranging from 0% to 57.9% in Summer and Winter, respectively. In addition, 4 rodents had a mixed *L. major* and *L. killicki* infection. The detection of the both *Leishmania* species parasites among this rodent by direct examination and PCR with such a high infection prevalence constitutes a strong evidence of its role as a reservoir host in these Leishmania species lifecycles.

Keywords *Ctenodactylus gundi*; *Leishmania major*; *Leishmania killicki*; RODENT RESERVOIR; CUTANEOUS LEISHMANIASIS

P4-079: *Leishmania infantum* INFECTION IN EXOTIC PETS LIVING IN METROPOLITAN AREA OF LISBON, PORTUGAL: WHAT DO WE KNOW?

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Leishmania infantum is a protozoan able to infect wild and domestic animals, causing a zoonotic infectious parasitic disease of worldwide concern. Nowadays, exotic animals, such as rodents, lagomorphs, and mustelids, that are proven to be susceptible to *L. infantum* infection, are kept as pets in many countries including Portugal. However, there is a lack of information available on the *Leishmania* infection and leishmaniosis in these exotic pets. In this work we aimed to: (i) determine *L. infantum* infection in rabbits, guinea pigs, chinchillas, and ferrets kept as pets living in the Metropolitan Area of Lisbon using IFAT; (ii) evaluate concomitant infection by other hemoparasites in blood smears; (iii) verify the possible impact on the relationship between animals and humans, under the "One Health" concept by analysing data collected in a questionnaire concerning risk factors. Prior to the beginning of this study, the experimental design was submitted to the evaluation of the Ethics Committee for Research and Teaching of the FMV, and owners were duly informed. Owners were also given an elucidative flyer and were asked to give written consent for blood sampling and to answer a questionnaire. This was composed of 35 questions about the pet, cohabiting animals, and how aware owners were about leishmaniosis. Blood samples were collected from 34 asymptomatic exotic pets: 24 rabbits, 6 guinea pigs,

2 ferrets, and 2 chinchillas. Antibodies anti-*L. infantum* were screened using Canine Leishmaniasis IgG IFA, Fuller Laboratories Fullerton, CA, USA kit (cut-off 1:40 and 1:80), and detection of other blood parasites was done through direct observation of Giemsa stained blood smears. When inconclusive results were obtained samples were tested by PCR and qPCR. No anti-*Leishmania infantum* antibodies were detected using IFAT nor hemoparasites were observed in blood smears. Although the results were all negative, the presence of parasites cannot be excluded once in AML, mild average monthly temperature throughout the year constitutes an ideal condition for *Phlebotomus* spp, namely *P. perniciosus*, *P. papatasii* and *P. sergenti*. Additionally, many of these pets are living in indoors in peri-urban areas although all respondents stated that they had never seen ectoparasites on their animals and the majority respect the regularity of deworming and vaccination schedules. In this first investigation regarding *L. infantum* infection in exotic pets in AML, the lack of available information regarding *L. infantum* infection and leishmaniasis in exotic pets and therefore its impact on the maintenance of the cycle of this parasite was also noted. Within the scope of One Health and Millenium Development goals, the control of *Leishmania* infection/leishmaniosis also relies on educating the veterinary community and animal keepers about this potential disease in exotic pets and further studies are needed.

Keywords *Leishmania infantum*; exotic PETS; RODENTS; LAGOMORPHS; MUSTELIDS

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P4-080: MOLECULAR AND SEROLOGICAL SURVEY OF *Leishmania infantum* IN WILD LEPORIDAE FROM MAINLAND PORTUGAL

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Leporids, either domestic or wild, can harbour a wide variety of infectious microorganisms. Wild leporids, are more exposed to several pathogens. Some of these are vector-borne such as *Leishmania*, a protozoan parasite transmitted by phlebotomine sand flies. The importance of wild rabbit as host of many of pathogens is still under debate and further studies are needed to acknowledge their role as pathogen reservoir and potential source for zoonotic transmissions. More recently, leporids have been identified as competent reservoirs of *Leishmania infantum*, being considered the source of infection that caused the largest human leishmaniosis outbreak registered in Europe to date. The objective of this study was to evaluate *L. infantum* infection in 170 wild leporids, using molecular and serological methods. Sixty-one wild rabbits (*Oryctolagus cuniculus algirus*) and 109 Iberian hares (*Lepus granatensis*) caught in different regions of mainland Portugal within the scope of the Action Plan for the Control of Rabbit Viral Haemorrhagic Disease + Coelho (Dispatch 4757/17 of May 31, funded by Fundo Florestal Permanente – Ministry of Agriculture, Portugal), were analysed. During necropsy, samples were taken from liver, spleen, lungs, bone marrow, mesenteric lymph node and skin of the ear. Data regarding age, sex, presence of other infectious agents and pathological changes displayed at *post-mortem* exam were also gathered.

Due to the lack of blood samples, lungs' samples from 18 wild rabbits and 45 hares were used to obtain exudates for the detection of anti-*L. infantum* antibodies with an adaptation of Canine Leishmaniosis IgG IFA, Fuller Laboratories Fullerton, CA USA kit (cut-off 1:40 and 1:80) Serum samples from 33 hare were also tested for anti-*L. infantum* antibodies. Using real-time PCR, *L. infantum* DNA was not detected in any of the 170 samples analysed. However, antibodies anti-*Leishmania* were detected in 11 out of 33 (33%) serum samples of hares tested. Although leporids are proven competent reservoirs of *L. infantum* even for human leishmaniosis outbreaks, until now there were no reports regarding *Leishmania* infection in wild Leporidae in Portugal. In this study the authors showed that despite no *Leishmania* DNA detection, the presence of antibodies clearly indicate that the animals were infected with trypanosomatids. The negative PCR results may be explained by i) a low amount of DNA in the tested organs that would failed detection; ii) the absence of visceral infection or by iii) representing past solved infections that let no lesions nor parasites detectable at *post-mortem* examination. The latter explanation, would point towards an ability of the immune system of the hare to control infection, favoring this species to act as reservoir of the parasite. However, it cannot be excluded that the detected antibodies may not be against *L. infantum* but against other member from Trypanosomatidae family that cross reacted namely *Trypanosoma nabiasi* transmitted by *Spilopsylus cuniculi.* To better understand the epidemiology of *Leishmania* infection in wild leporids further studies must be developed to complete this pioneer study.

Keywords *Leishmania*; IBERIAN HARE; WILD RABBIT; PCR; IFAT; PORTUGAL

Financing Action Plan Control RVHD +Coelho Ref. 2017014300001; FCT-CIISA, UIDB/00276/2020

P4-081: LABORATORY OBSERVATIONS OF *Nyssomyia antunesi* AND *Trichophoromyia brachipyga* (DIPTERA: PSYCHODIDAE: PHLEBOTOMINAE)

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Phlebotomines (Diptera: Psychodidae: Phlebotominae) comprise medically important insects due their role in the transmission of *Leishmania* parasites, the causal agents of leishmaniases. Among phlebotomine species of the Amazon region, *Nyssomyia antunesi* is regarded as a suspected vector of *L. (Viannia) lindenbergi* while *Trichophoromyia brachipyga* is a possible alternative vector of *L. (V.) lainsoni*. In this work, life tables of *Ny. antunesi* and *Th. brachipyga* were constructed by studying the immature stages under laboratory conditions. Phlebotomines were captured in the urban park Bosque Rodrigues Alves - Jardim Botânico da Amazônia (BRAJBA) in Belém, Pará State, Brazil, using CDC traps, subsequently fed on hamsters, and then separated by species in containers with wet filter paper as substrate for breeding. The development of the immature forms was carried out in an incubator under 27 ± 0.1 °C and > 90% humidity, with a diet based on bovine dessicated liver. Thirty females of *Th. brachipyga* laid 756 eggs (x = 37 ± 17), evolving to 359 (47.5%) L1, 183 (24,4%) L2, 141 (18,7%) L3, 113 (14,9%) L4, 64 (8,5%) pupae, and emerging 27 (7.5%) adults. Eleven females of *Ny. antunesi* laid 506 eggs (x = 60 ± 35), evolving 89 (17.6%) L1, 54 (10,7%) L2, 49 (9,7%) L3, 45 (8,9%) L4, 23 (4,5%) pupae, and emerging

14 (15.7%) adults. The total development (from oviposition to adult death) of *Ny. antunesi* took 71.5 ± 22.2 days while that of *Th. brachipyga* was 97.2 ± 35.3 days. Survival curves of both species showed that mortality decreased after the second larval instar and tended to constant values in the next instars/stages. This is the first study on the life cycle of *Th. brachipyga* and complements earlier studies carried out with *Ny. antunesi*. Present results improved knowledge on the bionomics of phlebotomines under laboratory conditions, particularly to those species potentially associated with *Leishmania* transmission.

Keywords TABLE OF LIFE; *Phlebotominae*; LEISHMANIASES; *Nyssomyia antunesi; Trichophoromyia brachipyga.*

Financing IEC/MS

P4-082: ECOLOGICAL INTERACTIONS OF SANDFLIES, *Leishmania* PARASITES AND RESERVOIRS IN AN ENDEMIC AREA OF CUTANEOUS LEISHMANIASIS (CALDAS-COLOMBIA)

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Phlebotomine Sandflies (Diptera: Psychodidae) include species recognized as vectors of *Leishmania* spp. This group of insects has a wide distribution worldwide with higher diversity in tropical regions. Sandflies have adapted to multiple blood sources that can affect relevant aspects of their life cycle or increase interactions with species known as possible reservoirs and thus influence the transmission dynamics of leishmaniasis. Identification of the blood sources and surveillance of parasite circulation in sandfly species plays an important role in epidemiological studies because it contributes to a better understanding of the natural transmission cycle and the development of strategies for disease control. This study aimed to determine the natural infection of sandflies with *Leishmania* spp and to characterize the blood sources in fed females. Sampling of sandflies was carried out monthly from November 2020 to October 2021 with CDC-type light traps installed both in the intra and peridomicile of six houses, for three consecutive nights. Additionally, a Shannon trap was installed in a peridomicile. We identified sandfly species according to morphological characters using the taxonomic key of Galati (2003). A sample of females was identified using head and spermathecae characters and after that, DNA was extracted from the remaining parts of the body. To investigate the presence of *Leishmania* sp DNA, female sandflies were grouped into pools

according to species. Engorged females were processed individually to identify the blood source. The presence of *Leishmania* was identified by conventional PCR using HSP70 gene as the target and for blood-feeding source identification PCR products of the vertebrate 12S rRNA gene were obtained and sequenced for Sanger sequencing. Blood-meal sources were inferred using blastn against a reference dataset containing the 12S rRNA sequences belonging to vertebrates with a distribution in South America (a potential feeding source for sandflies) and other conventional PCR targeting cytochrome b (*cyt*b) gene of vertebrates was also done. Positive PCR products were sequenced and compared with sequences deposited in GenBank using the BLASTN search. A total of 4,621 sandflies were collected, 76% of females. A total of 940 females of 15 species were grouped in 321 pools and processed. *Leishmania* sp was detected in 08 pools giving a minimum infection rate of 2.5%. The females engorged (n =70) corresponded to 20% of the samples; for 28 of them *cytb* was obtained and are being processed for blood source identification. Our results demonstrate the circulation of *Leishmania* sp. in the sandfly population of the study area and with the future characterization of the blood meal source obtained will provide knowledge on the vector-parasite-reservoir interaction in this endemic area, contributing to understand the feeding habits and other key ecological aspects of the eco-epidemiology of CL in this region.

Keywords SAND FLY; BLOOD SOURCE; NATURAL INFECTION

P4-084: EVALUATING THE DIVERSITY OF TRYPANOSOMATID PARASITES INFECTING BATS FROM DIFFERENT ECOREGIONS IN COLOMBIA

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Bats are one of the most abundant and diverse groups of mammals globally, and Colombia harbors 209 species. Additionally, bats are involved as reservoirs in parasite, virus and bacteria transmission cycles, making it relevant to establish their role in zoonotic transmission. Here, we aimed to detect the presence of trypanosomatids in bats captured in different ecoregions in Colombia, to identify them and to evaluate the diversity of bats and their associated trypanosomatids in four different habitats (natural savannas, riparian forest, terra firme forest and rice crops). We performed fieldwork in six localities using mist nets, and captured individuals were sedated, and blood and tissue samples were taken. Additional samples from collaborators were obtained for a total of 235 bat samples from different ecoregions. At the laboratory, we performed DNA extraction, amplified target sequences from the regions Cytochrome B and HSP70 in conventional PCR and send the amplified products to sequencing by sanger. We also performed a conventional PCR to amplify the gen Miniexon to corroborate the identification of the *Leishmania* specie. The obtained sequences were depurated and then we performed a Blastn to establish a preliminary identification. Sequences were aligned using MUSCLE and reference sequences from GenBank and build phylogenetic trees, one for the genus *Trypanosoma* and another for the genus *Leishmania*. Finally, we did a

rarefaction curve to evaluate the quality of the sampling, calculated the inverse Simpson index and the proportion of individuals to establish the diversity of bats in each habitat. As results, we found two of 235 bats infected with *Trypanosoma cruzi* TcI and one bat of the specie *Molossus molossus* infected with *Leishmania amazonensis*. For the first time, bats are reported as infected with *Leishmania* parasites in Colombia with the specie *Leishmania amazonensis* that has been reported in bats of Brazil and the other specie (*Leishmania mexicana*) that also belongs to the complex *Leishmania (L) mexicana* which infects bats in Mexico. Regarding the results of bat diversity, forests were the most diverse compared to the rice crops and the savanna, however these differences are not significant. In relation to the diversity of trypanosomatids collected in the four habitats, only two bats were infected with *T.cruzi*, the first collected in terra firme forest and the other collected in a refuge close to the house. The prevalence of infection with trypanosomatids in bats in Colombia is low compared to the infection found in other countries, and interestingly only trypanosomatids with medical importance were found in the sampled ecoregions. Infected bats were insectivorous, which can explain the transmission route for *Trypanosoma cruzi*. All positive specimens were collated in proximity to human dwellings which poses a higher risk of infection with trypanosomatids of medical importance.

Keywords *Trypanosoma cruzi* TCI; *Leishmania amazonensis*; HSP70; CYTB; HOSTS

Financing Proyecto Semilla Universidad de los Andes and Scholarship Bat Conservation International proyect “Effect of transformed ecosystems on bat and trypanosomatid parasites diversity in the Colombian Llanos”

P4-086: FIRST REPORT OF TRANSPLACENTALLY TRANSMISSION OF *Leishmania* sp. IN NATURALLY INFECTED PREGNANT CATS

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Leishmaniosis is worldwide distributed, *Leishmania infantum* causing the disease in several species, like humans and dogs in Latin-America, including Brazil. There are several evidence that cats are also infected by *L. infantum* in Brazil. The principal way of transmission between animals is throughout sand fly (*Lutzomyia longipalpis*) byte, although other forms of transmission were related, including venereal and vertical transmission in humans and dogs. To the best of our knowledge, these alternative transmission ways were not related in cats. The goal of this study was to evaluate the possibility of transplacentally transmission of *Leishmania* sp. in cats. Four pregnant cats from Belo Horizonte municipality of Minas Gerais state, Brazil, submitted to ovary salpinge-hysterectomy during a public health project for population control, have their uterus submitted to histopathological exams, blood collected for serum detection of *Leishmania* spp. antibodies (ELISA) using plates sensibilized with 100 ng of rKDDR. Plus antigen. Vulvovaginal secretion of female cats was gently collected by swabbing the vulvovaginal surface with cotton tips of the swabs moistened with sterile saline solution, and fetus tissues (liver and spleen for 30- and 40-day fetus and whole body

for 25-day fetus) were submitted to conventional PCR for detection of *Leishmania* sp. using the forward 5'CTTTTCTGGTCCCGCGGGTAGG3' and reverse 5'CCACCTGGCCTATTTTACACCA3' primers. The results were: cat #1 with normal histology, ELISA negative, genital secretion negative and 1 of 2 40-day fetus positive; cat #2 with moderate endometritis, ELISA positive, genital secretion positive and 1 of 4 40-day fetus positive; cat #3 with normal histology, ELISA negative, genital secretion negative and 2 of 4 30-day fetus positive; cat #4 with normal histology, ELISA negative, genital secretion positive and 3 of 5 25-day fetus positive. In summary, all pregnant cats, despite of their serological and genital secretion molecular status, carried at least one PCR-positive fetus inside uterus (8/13 - 61.5%), despite of signs of endometritis (1/4 female – 25%). Although only four pregnancies examined, the finding of all of them with at least one transplacentally infected fetus seems that this kind of vertical transmission may be highly frequent in cats. In conclusion, *Leishmania* sp., probably *L. infantum*, can be transplacentally transmitted to fetuses in pregnant cats.

Keywords FELINE LEISHMANIOSIS; GENITAL INFECTION; FELINE GENITAL TRACT; VERTICAL TRANSMISSION; FETUS CONTAMINATION

P4-087: FIRST REPORT OF *Leishmania* sp. IDENTIFICATION IN GENITAL SECRETIONS OF NATURALLY INFECTED CATS

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Leishmaniosis is worldwide distributed, *Leishmania infantum* causes the disease in humans and dogs in Latin-America, including Brazil in where there are several evidence that cats are also infected by *L. infantum*. The presence of *L. infantum* in genital secretions of naturally infected male and female dogs and venereal transmission of the parasite from dog to bitch are related and already reported. The goal of this study was to evaluate by molecular tests, the presence of *Leishmania* spp. in genital secretions of cats from Belo Horizonte municipality of Minas Gerais state, Brazil, which was not reported in cats before. Vulvovaginal or preputial secretions and genital organs from 89 female and 64 male cats were evaluated. Genital secretions were obtained by gently swabbing the vulvovaginal or preputial surface with cotton tips moistened with sterile saline solution. Serology (ELISA) of the cats was performed for anti-*Leishmania* spp. antibodies detection by using plates sensibilized with 100ng of rKDDR-Plus antigen. Conventional PCR of genital secretions was performed employing the primers: F-5'CTTTTCTGGTCCCGCGGGTAGG3' and R-5'CCACCTGGCCTATTTTACACCA3'. ELISA was reagent in 33.7% female and

43.8% male cats; genital secretions were PCR-positive in 31.5% female and 68.5% male cats; ELISA and PCR were positive in 33.3% female and 7.1% male cats. These results demonstrated, for the first time, that male and female genital secretions may contain *Leishmania* spp. gDNA in naturally infected cats, probably *L. infantum*, and suggest that these animals may be potentially infected during mating, probably through venereal transmission. In addition, all these findings, besides transmission during parturition, require attention and further studies since this work was able to demonstrate that cats naturally infected by *Leishmania* spp. can release the parasite in their genital secretions.

KEYWORDS FELINE LEISHMANIOSIS; GENITAL INFECTION; GENITAL SECRETION; FELINE GENITAL TRACT

P4-088: VISCERALIZATION OF *Leishmania infantum* IN CATS

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Leishmaniasis in cats has been gaining attention due to the high frequency of the parasite's presence in skin cytology associated with lesions such as alopecia, ulcers and skin nodules. However, the visceralization of *Leishmania* and the lesions caused in visceral organs in cats are still poorly understood. Therefore, the objective of this study was to evaluate the occurrence of *Leishmania* in the skin of infected cats, as well as the occurrence of the parasite and the lesions in visceral organs that indicate the visceralization of *Leishmania infantum* in these animals. In this study, data from 20 leishmaniasis-positive cats were analyzed, from a disease prevalence study carried out in Teresina, state of Piauí, Brazil, diagnosed by direct investigation of the parasite in bone marrow, popliteal lymph node and skin. The parasite was isolated from 20 animals and the species found was *L. infantum*, which was identified by polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) and sequencing. All 20 cats underwent clinical evaluation and blood collection for serum urea, creatinine, total protein, albumin, globulin and alanine aminotransferase (ALT) measurements. Twelve of the 20 infected cats underwent xenodiagnosis to verify the presence of the parasite on the skin and the ability to infect the vector, and five were euthanized and spleen, liver and kidney tissue collected for histopathological diagnosis. In the clinical

evaluation, it was observed that 65% of the animals had skin lesions (alopecia 55%, ulcerative lesions 40% and skin nodules 25%). The animals also had lymphadenomegaly 65%, weight loss 40%, uveitis 15%, blepharitis 10%, ocular discharge 5%, blindness 5% and 10% were asymptomatic. In the xenodiagnosis 8/12 (67%) were able to infect the vector and the frequency of infected insects per animal ranged from 21.9% to 94.4%, mean 31.0%, indicating a high frequency and parasite load of *Leishmania* in the cat skin. In the evaluation of organs such as kidney and liver by means of biochemical quantifications, none cat (0/20) had elevated creatinine, only 2 (10%) had high urea and 1 (5%) had high ALT. Hypoalbumin was present in 7 (35%), elevated globulin in 15 (75%) and increased total protein in 15 (75%). As for the histopathological findings, it was observed in the spleen: hyperemia in the five animals (100%), white pulp hyperplasia in 80% and red stern hyperplasia 20%. Liver: degeneration 80%, hyperemia 60%, lymphoplasmacytic hepatitis 40% and lymphocytic hepatitis 20%. Kidneys: hyperemia 80%, proliferative glomerulonephritis 40%, membranoproliferative glomerulonephritis 20% and interstitial nephritis 20%. *Leishmania* sp. was observed in only one spleen sample. The lesions found in the liver, spleen and kidney ranged from mild to moderate. Severe lesions were not found. With the findings of this study, it is concluded that the visceralization of *L. infantum* in the cat was confirmed, but it did not cause serious injuries in the evaluated visceral organs. It was possible to verify the high predilection of *L. infantum* for the skin of the cat, where it causes multiple, frequent and serious lesions, besides facilitating the infection of the vector.

Keywords LEISHMANIASIS; FELINE; PATHOGENESIS; INJURIES

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P4-090: EVALUATION OF RODENT INFECTIVITY AS RESERVOIR HOSTS IN THE TRANSMISSION CYCLE OF AMERICAN TEGUMENTARY LEISHMANIASIS IN PERNAMBUCO STATE, BRAZIL

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Leishmaniases are a complex of diseases caused by parasitic protozoa (Kinetoplastida: Trypanosomatidae) of the genus *Leishmania*, which are transmitted to animals and humans by phlebotomine sand fly vectors. One of the gaps related to American Tegumentary Leishmaniasis (ATL) eco-epidemiology associated with *Leishmania (Viannia) braziliensis* is related to identification of reservoir hosts and sand flies that keeps the transmission cycle. Amaraji, municipality of Zona da Mata of Pernambuco state, show a significant incidence of ATL. This study aimed to characterize the infectivity of wild and synanthropic rodents to *L. (V.) braziliensis* as reservoirs involved in maintaining the zoonotic cycle in the region, through the diagnosis of natural infection detected by qPCR (quantitative Reaction Polymerase Chain); xenodiagnoses using fed sandflies (*Lutzomyia longipalpis* and *Lutzomyia whitmani)* on infected rodents; and evaluation of exposure to interrupt transmission. Experimental study conducted between may/2012 and august/2014 was captured 638 rodents of 11 various species, with a predominance of *Nectomys squamipes* 38.3% (245/638), and *Rattus rattus* 23.2% (148/638). They were mark with microchips 603 rodents, and performed 394 recaptures. DNA samples were obtained from skin and blood of rodents every capture / recapture. In 176 (29.2%) was detected rodents'

infection. 51 xenodiagnosis were performed (46 using *Lu. whitmani* and 5 using *Lu. longipalpis*), where an infected 72.58% (1400/1929) of sand flies. No differences were identified as the vector species. Rodents were infectious to vectors regardless of the load parasite of infection. It was observed decrease in parasite load of laboratory rodents. Natural infection by *Leishmania (Viannia) braziliensis* in rodents indicate that *N. squamipes* and *N. lasiurus* act as primary reservoirs and *R. rattus* as secondary reservoir in transmission cycle of ATL in the region.

Keywords. *Leishmania braziliensis* - PARASITOLOGY. DISEASE RESERVOIRS

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P4-091: GENETIC VARIABILITY OF *Triatoma maculata* (HEMIPTERA, REDUVIIDAE, TRIATOMINE) OF DIFFERENT ECOTOPES IN ENDEMIC AREAS OF CHAGAS DISEASE IN VENEZUELA

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Triatoma maculata is the vector of *Trypanosoma cruzi,* the causative agent of Chagas disease (Ech), has been considered a wild species typical of palms, dry trees, fences and bird nests. This vector is adapting to domestic habits, with the parasite positivity, representing a risk for disease transmission. Genetic variability and intraspecific variation of this species can be instrumental in vector control and surveillance. This study attempts to analyze comparatively the variability of mitochondrial cytochrome b gene (*Cyt* b) and the 16S ribosomal RNA gene (16S rRNA) of *T. maculata*, from different localities and ecotopes of endemic regions in Venezuela, and its possible relationship to the habit of the vector. Active and passive capture of *T. maculata* was performed, an a total of 176 specimens were collected in homes (D), peridomiciliary (PD) and wild (S) habitats in villages of different Venezuelan states: Miranda, Anzoátegui, Bolivar, Monagas, Portuguesa and Sucre. DNA was isolated from the legs of specimens and used for amplification of the 16S rRNA and *Cyt* b genes. Sequencing of PCR product was carried out and subsequent PCR-RFLP analysis was performed to establish the variability of the markers and its association to the different ecotopes. *T. maculata* located in D ecotope showed moderate genetic

variability (Fst 0.052 to 0.12) and the rate of migration revealed independent gene flow (Nm> 1) in the region of study. In different regions of Venezuela, *T. maculata* in D ecotopes showed less genetic variation in the 16S rRNA molecular marker than Cyt-b gene with respect to the S ecotope, making discriminating variation ecotopes. This genetic variation could be interpreted as a risk factor in the transmission of Ech. Thus, it is necessary to study the genetic structure of S foci, their possible dispersion routes and the epidemiological risk they represent.

Keywords CHAGAS DISEASE; TRIATOMA; GENETIC VARIATION; GENETIC POLYMORPHISM

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P4-092: FIRST REPORT OF *Leishmania infantum* IN MILK IN A NATURALLY INFECTED BITCH FROM BRAZIL

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Visceral Leishmaniasis is a cosmopolitan disease, caused by *Leishmania infantum* affecting humans and several animal species. Among animals, dogs are the main known reservoir and play an important role in transmission cycle through infected sandflies, particularly *Lutzomyia longipalpis* in Brazil. Dogs, besides reservoirs, are also susceptible to infection and often get sick and even die. The amastigote forms spread through in the body and was already described in the bone marrow, blood, spleen, liver, kidneys, lungs, among others. More recently was reported in the mammary glands of bitches, although never been identified in milk. This report describes a 6-year-old female Keeshond that given birth to four puppies, three stillborn and one full-term, healthy and nursing, 20 days before biological samples collection. The bitch was serologically positive for *Leishmania* spp. in ELISA and RIFI tests and with a parasite load in bone marrow of 822,739.44 DNA copies/mL by *q*PCR. She was apparently healthy and with normal laboratory tests, except for an elevation of globulins (5.3 g/dL). Milk was collected by milking from different breasts, stored in sterile microtubes and sent for molecular tests - conventional PCR, *q*PCR and PCR-RFLP in an attempt to detect the presence of *Leishmania* spp. DNA, quantify the parasite load and characterize its species. For conventional PCR, kinetoplast DNA - kDNA, was used in the following primers: 150 - 5' (C/G) (C/G) (G/C) CC(C/A) CTA T(T/A) T TAC ACC AAC CCC 3' and 152 - 5' GGG GAG GGG CGT TCT GCG AA

3', generating a 120bp fragment. *Leishmania* spp. gDNA was detected and parasite load obtained was 683.686,87 DNA copies/µL (Probe-based qPCR targeting kDNA minicircle - Tecsa® Laboratories). The specie was characterized through PCR-RFLP by using the Internal Transcribed Spacer - ITS1 as target with an amplicon of, approximately 350bp amplified with the following primers: L5.8S: 5'- TGA TAC CAC TTA TCG CAC IT -3' and L5.8SR: 5'- AAG TGC GAT AAG TGG TA -3', digest with HaeIII restriction enzyme which allowed the identification of *L. infantum*. This is the first report of the presence of *Leishmania infantum* in milk from a lactating bitch. Further studies should be conducted to assess the importance of this finding in the epidemiology of CanL.

Keywords CANINE LEISHMANIOSIS; MILK; PCR; *Leishmania infantum*

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