

Abstracts Index

Oral Presentations	5
Gaps in Phlebotomine distribution and their taxonomic implications	5
Phlebotomine DNA barcodes and new records for Argentina.....	6
An overview of the Phlebotomine sandflies of Madagascar.....	7
New data about the genus <i>Idiophlebotomus</i>	8
A molecular systematics of the Psychodopygina (Diptera; Psychodidae) inferred from ribosomal and mitochondrial DNA.....	9
Immune events triggered in host skin by a <i>Leishmania</i> -infected sand fly bite govern pathogenesis of leishmaniasis	10
<i>Lutzomyia longipalpis</i> salivary gland extract modulates <i>Leishmania enriettii</i> infection in <i>Cavia porcellus</i>	11
Attractiveness of golden hamster (<i>Mesocricetus auratus</i>) to <i>Lutzomyia longipalpis</i> (Diptera:Psychodidae) before and after infection with <i>Leishmania</i> (<i>Leishmania</i>) <i>amazonensis</i>	12
A hamster model of defective sand-fly transmission may explain the occurrence of canine <i>Leishmania</i> seroreactors without evidence of infection in endemic areas of zoonotic visceral leishmaniasis	13
<i>Leishmania mexicana</i> amastigotes of various origin: proteomic and developmental comparison...	14
Antibody response to Toscana virus, sandfly fever Sicilian virus and <i>Phlebotomus perniciosus</i> saliva in cats naturally exposed to sand fly bites	15
Host preference of the sand fly <i>Phlebotomus perfiliewi</i> in northern Italy	16
An overview of Brazilian sand flies: Almost a century of scientific data and how fast are we moving on to uncover which are the truly vectors?	17
Ecology and biting behavior of <i>Phlebotomus pedifer</i> in a cutaneous leishmaniasis hotspot in southern Ethiopia	18
Influence of paromomycin resistance on <i>Leishmania</i> fitness inside the sandfly vector.....	19
Miltefosine resistance in <i>Leishmania</i> : What do sand fly infections teach us?.....	20
Antibodies against sand fly saliva: markers of host exposure to sand fly vectors.....	21
Repeated sand fly bites have effect on <i>Leishmania</i> infection in BALB/c mice	22
Visceral leishmaniasis transmission in India: The role of xenomonitoring in a post-elimination setting	23
Are Arvicanthis and Mastomys Sub-Saharan reservoirs for <i>Leishmania major</i> and <i>L. donovani</i> ? Xenodiagnoses by <i>P. duboscqi</i> and <i>P. orientalis</i>	24
<i>Leishmania infantum chagasi</i> gene expression journey inside <i>Lutzomyia longipalpis</i> , the main vector of visceral leishmaniasis in Brazil	25
How to make a perfect cutaneous leishmaniasis outbreak in the Americas	26
Ecology of sand flies (Diptera: Psychodidae: Phlebotominae) in the municipality of Campo Grande, MS, Brazil	27
Diversity, species composition and natural infection of sand flies vectors of cutaneous leishmaniasis along an altitudinal gradient on the Northwestern slopes of the Ecuadorian Andes. .	28
Sand Fly Behavior: Much More Than Weak-Flying	29

Bionomy of the phlebotomine sandflies vectors and ecoepidemiological modulation of leishmaniasis epidemic outbreaks in America, Africa, Europe and Asia	30
Sandfly-borne phleboviruses circulating in Portugal: five and still counting	31
Updates on the distribution, diversity and abundance of sand flies (Diptera: Psychodidae) in Romania	32
Multi-scale influences on microbial sandfly community structure in Amazonia	33
Impact of anthropic activities on the diversity and distribution of sandflies species in the eastern region of Caldas, Colombia	34
Phlebotomine sand flies and leishmaniasis in Oman	35
Diversity and ecology of phlebotomine sand flies (Diptera: Psychodidae) in the Quang Ninh province, a new update for Vietnam	36
Altitude and hillside orientation as main factors of population structuring of <i>Phlebotomus ariasi</i> , vector of <i>Leishmania infantum</i>	37
What we have learned from sandfly-borne Phlebovirus researches in old world?	38
Phlebotominae (Diptera: Psychodidae) and Biomes in the State of Mato Grosso do Sul, Brazil	39
Sand Fly-borne Phleboviruses in the Balkan Countries.....	40
Field evaluation of transfluthrin treated military materials against Phlebotomine sand flies (Diptera: Psychodidae) in Thailand.....	41
Attractiveness of a controlled release system with 1-hexanol to <i>Nyssomyia neivai</i> (Diptera: Psychodidae) in wind tunnel	42
<i>Phlebotomus perniciosus</i> biological adaptation under laboratory conditions	43
A comparative study of three trapping methods for indoor sampling of <i>Phlebotomus argentipes</i> in the Indian subcontinent	44
Indoor residual spraying for the control of <i>Phlebotomus argentipes</i> in the Indian subcontinent: a review of the available evidence	45
Bacterial infection and innate immune responses in sand fly larvae midgut	46
Zooprophylaxis: Impact of traditional breeding rabbits in man-made underground holes located in the peri-domestic areas on the transmission of zoonotic cutaneous leishmaniasis in Central Tunisia	47
Laboratory reassessment of the anti-feeding effect of Scalibor® deltamethrin collar against the sand fly <i>Phlebotomus perniciosus</i> in dogs confirms elevated and sustained efficacy for one year following treatment	48
Yellow-related proteins in saliva of <i>Phlebotomus orientalis</i> : amine-binding and antigenic properties	49
Population structure of two sand fly genomes: <i>Phlebotomus apatasi</i> and <i>Lutzomyia longipalpis</i> ...	50
<i>Sergentomyia schwetzi</i> : salivary gland transcriptome, proteome and salivary enzymes	51
Bacterial diversity of wild-caught <i>Lutzomyia longipalpis</i> (a vector of Visceral Leishmaniasis) under distinct physiological conditions, by metagenomics and cultivable analysis.	52
MALDI-TOF mass spectrometry: useful tool for species identification and blood meal analysis of Phlebotomine sand flies	53
Posters Presentations	55
Poster Session 1	56

Central Asian rodents as model animals for leishmaniasis: susceptibility to <i>Leishmania major</i> and infectiousness to <i>Phlebotomus duboscqi</i>	56
Ultrastructural studies on Alcube virus, a new sandfly-borne virus isolated in Portugal.....	57
Adaptive gene-loss coupled to <i>Leishmania infantum</i> expansion in Brazil.....	58
Development of <i>Leishmania</i> of the subgenus <i>Mundinia</i> in sand flies and guinea-pigs.....	59
Genetic Structure Analyses of <i>Lutzomyia longipalpis</i> and <i>Lutzomyia cruzi</i> using Next-Generation Sequencing.....	60
Molecular taxonomic identification and genetic relationships among <i>Lutzomyia longipalpis</i> complex and closely related species inferred from cytochrome c oxidase subunit 1.....	61
A robust phylogeny of the subgenus <i>Paraphlebotomus</i> inferred by large-scale sequencing (RAD-seq).....	62
Endemism Areas of Phlebotominae in the Upper Paraguay River Basin, Mato Grosso do Sul, Brazil.....	63
Impact of productive subsistence activities on the abundance dynamic of <i>Nyssomyia whitmani</i> , a <i>Leishmania braziliensis</i> vector in Misiones, Argentina.....	64
Blood feeding of <i>Sciopemyia microps</i> (Mangabeira, 1942) and <i>Sciopemyia sordellii</i> (Shannon & Del Ponte, 1927) in caves of Minas Gerais state, Brazil.....	65
A historical overview on sand fly distribution and epidemiology of human and animal leishmaniasis in Eastern Europe.....	66
mtDNA genetic variability of <i>Lutzomyia longipalpis</i> from Uruguay highlights its invasion route to the country.....	67
Diversity of the phlebotomine fauna (Diptera: Psychodidae) in an area of occurrence of American cutaneous leishmaniasis, State of Acre, Brazilian Western Amazonia.....	68
Human seroprevalence of Toscana virus and Sandfly fever Sicilian virus in Setúbal district, Portugal.....	69
Phlebotomine sand fly survey in Algarve region, south of Portugal, 2018: molecular screening of <i>Leishmania</i> and <i>Phlebovirus</i> , and blood meal identification.....	70
Presence of <i>Lutzomyia longipalpis</i> in Brazilian areas without cases of visceral leishmaniasis.....	72
Ecological study and risk factors for the transmission of American Cutaneous Leishmaniasis(ACL) in conservation areas - Paraná – Brazil.....	73
Ecological niche of <i>Lutzomyia longipalpis</i> (diptera: Psychodidae), vector of visceral leishmaniasis, in the state of Espírito Santo, Brazil.....	74
Survey of sandfly fauna in areas of transmission of American Cutaneous Leishmaniasis (ACL), evaluating abundance, diversity and equitability indices under influence of hydroelectric plants in the Paranapanema River, Paraná.....	75
Baseline sand fly data and monitoring of cutaneous leishmaniasis in an endemic area close to the Toachi-Pilaton hydroelectric dam: Joint initiative between HIDROTOAPI and LEMMT-USFQ..	76
Leishmaniasis in Galapagos: Past reported cases and, entomological data to address if local transmission is possible.....	77
Leishmanicidal activity of Ishpink and Moringa.....	78
Poster Session 2.....	79
Multi-scale environmental influences on sandfly community structure in Amazonia.....	79

Phlebotomine in transitional area, Chaco, Argentina. Meteorological variation.....	80
Seasonal distribution of <i>Lutzomyia longipalpis</i> in Santo Tomé, Corrientes, Argentina.....	81
Nictemeral rhythm of sand flies (diptera: psychodidae) in campo grande, Mato Grosso do Sul, Brazil.....	82
Where does <i>Nyssomyia whitmani</i> breed? Habitats and conditions that favour the vector in rural areas of Misiones, Argentina	83
Aspects of the phlebotomine fauna (Diptera: Psychodidae) and of the infection by <i>Leishmania</i> spp. in an area of high incidence of Cutaneous Leishmaniasis in Rio Branco, Acre	84
Spatial distribution of immatures and adults of <i>Lutzomyia</i> spp. in a periurban focus of leishmaniasis in the Caribbean region of Colombia	85
Emergence of Toscana virus and other sandfly-borne phleboviruses in arid bio-geographical areas of central Tunisia	86
Irrigation in the arid regions of Tunisia impacts the abundance of sand flies' population and <i>Leishmania infantum</i> infection.....	87
Natural infection of <i>Lu. longipalpis</i> (Cembrene-1) with <i>Leishmania infantum</i> in a VL focus of the East region of São Paulo State, Brazil	88
Detection of DNA of <i>Leishmania infantum</i> in <i>Nyssomyia intermedia</i> in a new focus of visceral leishmaniasis in São Paulo State, Brazil. What is the role of this sandfly in VL transmission?	89
Seasonal assemblage of Phlebotominae species, Tucumán, Argentina.	90
Distribution, Abundance and Infection of <i>Lutzomyia longipalpis</i> , vector of Visceral Leishmaniasis in three Municipalities of the Department of Caaguazú, Paraguay	91
Sandflies fauna (Diptera: Psychodidae) in a border area of Paraguay and the influence of environmental variables on the vector density of <i>Lutzomyia longipalpis</i>	92
Checklist and distribution maps of the phlebotomine sandflies of Bulgaria	93
Field collection of sand flies as one of the funded projects of local participatory budget in Torrelodones municipality (Madrid), Spain.....	94
Harnessing student citizen science in and around the classroom to study sand fly populations in Madrid (Spain)	95
Ecological and seasonal aspects of sand fly populations in Austria	96
Ecological niche modelling of <i>Nyssomyia trapidoi</i> and <i>Nyssomyia ylephiletor</i> , vectors of <i>Leishmania</i> , with comments on their geographical distribution	97
VectorBase: A bioinformatics resource for invertebrate vectors and other organisms related with human diseases.....	98
Multi scale environmental determinants of <i>Leishmania</i> vectors in urban and rural context, Argentina.....	99
Identification of vectors and possible vectors of leishmaniasis in north region of Ecuador	100
Evaluation of a pan- <i>Leishmania</i> SL-RNA qPCR assay for parasite detection in laboratory and field collected sand flies.	101
List of Participants	102

Oral Presentations

Gaps in Phlebotomine distribution and their taxonomic implications

Eunice A. B. Galati¹ and Fredy Galvis-Ovallos¹

¹Epidemiology Department, Public Health School, São Paulo University – São Paulo, Brazil.

*Corresponding author: egalati@usp.br

The objective of this approach was to analyze the pattern of geographical occurrences of species or groups of species of American sandflies belonging to three genera and to identify the gaps in their distribution which may have some implications for their taxonomy. A database of *Deanemyia*, *Pintomyia* and *Trichophoromyia* species was constructed. Data of the species were obtained by bibliographic research online, of the Rene Rachou-Fiocruz collection and personal information. Geographical data were obtained from the IBGE and ESRI platform. The distribution of the species was mapped according to information relating to the first administrative level of each country, and for Brazil, the second administrative level. The system of WGS 1984 geographic coordinates was adopted. The ecoregions adopted follow those of the US Environmental Protection Agency (EPA). The distribution of the *Pintomyia* was presented at subgenera level and series of species. A total of 127 species were analyzed. *Deanemyia* (05 species), *Trichophoromyia* (44) and *Pintomyia* (78), distributed according to the subgenera *Pintomyia* (8) and *Pifanomyia* (70). *Deanemyia*, although consisting of few species, is found in the Amazonian ecoregions: the moist forests of the Brazilian and Guyana shields and the Amazonian and Coastal lowlands, and the ecoregions of the Cerrados and Chaco, showing a discontinuous distribution possibly because this group is one of the oldest and may therefore have disappeared from some areas or because its species are troglodytes or troglobiont and their cave environments have been insufficiently studied. *Trichophoromyia* is predominantly present in the Amazonian ecoregions. It occurs in some of the Andean ecoregions and few species have been registered in the Cerrado/Atlantic forests and one of them in Central America. In Brazil, its distribution is found predominantly in Amazonian irregular plains and piedmont, Amazonian and Coastal lowlands and the Brazilian shield of moist forests. The *Pintomyia* subgenus presents a wide distribution, from Panama to Northern Argentina. In South America, it occurs throughout the several ecoregions, excepting the Western Dry Chaco. Discontinuous areas were detected in the distribution of *Pi. Fischeri*. For *Pi. damascenoi*, there is a clear gap between its distribution in the Amazonian ecoregions and the Caatinga, Southern Cerrados and southwestern Atlantic forests. *Pifanomyia* is distributed throughout the Caribbean basin, from the south of Mexico to Panama and in South America. The Serrana series is present in the Caribbean region as one existing species and although widely distributed in South America it is predominant in the Andean ecoregions. The Townsendi series occurs in the Caribbean, but only in fossil records. In South America its distribution is restricted to the Northern Andean Highlands. The Evansi series is well distributed from the south of Mexico to Panama and in South America, mainly in the Andean ecoregions. In Amazonia it has been registered in the Brazilian Shield Moist Forests close to the border with the Cerrados. Only *Pi. nevesi* occurs in Brazil where its distribution is restricted to the SW Brazilian Shield Moist Forests, forming an arc between this ecoregion and the Cerrados. Its occurrence in the Caatinga should be checked. The Verrucarum series is distributed in the Andean ecoregions (Northern Andean highlands and Central Andean/Puno Highlands) and also in Mexico, leaving a clear gap in its distribution. The Pia series has an Andean distribution similar to that of the Verrucarum series and has also been registered in Central America. The Pacae series is restricted to the Amazonian ecoregions: Brazilian and Guyana Shield Moist Forests and Amazon and coastal lowlands. The Monticola Series occurs in Atlantic forests and Cerrados, but has been registered in Central High Andes/Puno, thus showing a clearly disrupted distribution. Species have a close relationship with others of the same clade with respect to their morpho-bio-ecological characters. Thus when specimens of a particular species are identified it is strongly recommended that its distribution should be checked to ensure that there is no conflict regarding the distribution of the species. When there is a conflict, it is generally expressed in terms of geographical gaps. In the classification of a particular species it is recommended that the existence of gaps should be verified as regards the other species of the genus, subgenus, or species series to which was included. The presence of gaps may be a result of a misidentification of the species or its classification as belonging to a group which does not present the closest phylogenetic relation.

Phlebotomine DNA barcodes and new records for Argentina

SL Moya^{1,2*}, MG Quintana^{1,2,3}, A Pech-May^{1,2}, M Manteca Acosta⁴, D Lamattina¹, MG Giuliani¹,
OD Salomón^{1,2}, DJ Liotta^{1,5}

¹Instituto Nacional de Medicina Tropical (INMeT), ANLIS “Dr. Carlos G Malbrán”, Puerto Iguazú, Misiones, Argentina

²Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)

³Instituto Superior de Entomología “Dr. Abraham Willink”, San Miguel de Tucumán, Tucumán, Argentina

⁴Centro Nacional de Diagnóstico e Investigación en Endemo-epidemias (CeNDIE), ANLIS “Dr. Carlos G Malbrán”, Buenos Aires, Argentina;

⁵Laboratorio de Biología Molecular Aplicada (LaBiMAP-FCEQyN-UNaM), Posadas, Misiones, Argentina

*Corresponding author: sofialorian@gmail.com

Keywords: DNA Barcode, Cytochrome C Oxidase subunit I, Argentina.

Correct species identification is critical for any taxonomic group of interest, and particularly for those presenting challenges related to the existence of intraspecific phenotypic variation, cryptic species, and morphologically indistinguishable females of closely related species. Therefore, besides the still required traditional taxonomist expertise, molecular markers have been used to perform evolutionary systematics and more recently to contribute in the field of alpha taxonomy as is the proposal of the International Barcode of Life initiative. Despite the criticized choice of the mitochondrial marker, the performance of cytochrome C oxidase subunit I (COI) gene fragment was evaluated for phlebotomine fauna from different Latin American countries showing high efficiency with few exceptions generally related to recently divergent species. Therefore, our aim is to initiate a DNA barcode library and to evaluate its performance as a complementary tool for the morphological-based identification method for the Argentinian phlebotomine fauna, currently counting 13 genera and 38 species, including 6 proven vectors of visceral and cutaneous leishmaniasis agents. Once identified, sixty-one phlebotomine were chosen for DNA extraction with commercial kits from wings and legs previously dissected, the rest of the body was clarified and stored as a voucher. In addition, sixty-two archived samples were also included derived from projects in which DNA was extracted from thorax and abdomen, so no voucher was kept, and in some cases, the identification was checked again post-analysis. A 527 bp COI sequence was obtained for 123 specimens belonging to 19 species with the primers LCO1490 and HCO2198. Sequences alignment was performed using Clustal-W in MEGA v.7, as well as the estimation of K2p genetic distances, intra and inter-species, which we analyzed in order to evaluate the presence of a barcode gap. The resulting Neighbor Joining dendrogram identified 18 molecular taxonomic units (MOTUs) with high bootstrap support values ($\geq 99\%$) consistent with the species morphologically identified, except for *Nyssomyia whitmani* and *Ny. neivai* given their low inter-specific genetic distance (2.8%). The last two species belong to a group with evidence of introgression and low congener divergence showing a limit to the effectiveness of the barcode tool. Nevertheless, barcoding helped to solve particular misidentifications and allowed the association between females and males among morphologically similar species of some genera, e.g. *Brumptomyia* and *Psathyromyia*. In conclusion, barcoding made possible discrimination among these phlebotomine species with 95% efficiency. This study also reported new species for Argentina: *Br. cunhai*, *Br. nitzulescui* and *Br. ortizi*, and generated the first barcodes for *Martinsmyia alphabetica*, *Ny. neivai*, *Pa. lanei*, *Pintomyia pessoai*, *Cortelezzii* complex and *Shannoni* complex. The availability of DNA barcodes from different geographical regions allows comparisons within and between taxonomic groups that may resolve the status of some species or reveal hiding diversity of others, in both cases setting the direction of further studies towards an integrative taxonomic approach.

An overview of the Phlebotomine sandflies of Madagascar

Fano José Randrianambinintsoa*¹, Vincent Robert², Nicole Léger¹ & Jérôme Depaquit¹

¹ Université de Reims Champagne-Ardenne, ANSES, EA7510 « ESCAPE » - USC ANSES « transmission vectorielle et épidémiologie de maladies parasitaires (VECPAR) », 51, rue Cognacq-Jay, 51096 Reims – France

² MIVEGEC (IRD 224-CNRS 5290-Université Montpellier1), IRD, BP 64501, 34394 Montpellier, France

*Corresponding author: fano-jose.randrianambinintsoa1@univ-reims.fr

Keywords: inventory, morphological and molecular taxonomy, Madagascar, Indian Ocean.

The Malagasy sandfly fauna remained largely unexplored according to the lack of autochthonous transmission of leishmaniasis in Madagascar. Inventories have been carried out since the 2000's. Up to now, 20 species are already described. They belong to three genera (*Phlebotomus*, *Sergentomyia* and *Grassomyia*) and to six endemic subgenera (*Madaphlebotomus*, *Vattieromyia*, *Trouilletomyia*, *Ranavalomyia*, *Riouxomyia* and *Davidsonomyia*). We discuss the morphology of these species and their affinities with continental taxa at the light of both morphological and molecular data. Except the doubtful record of *Grassomyia squamipleuris*, all the Malagasy species are endemic. Little is known about the biology of Malagasy sandflies. A few of them are anthropophilic but their vectorial competence for *Leishmania* spp. remains unknown.

New data about the genus *Idiophlebotomus*

M. Loyer*¹, M. Nacri¹, F. Randrianambinintsoa¹, F. Gay² & J. Depaquit¹

¹ EA 7510 ESCAPE - USC ANSES VECPAR, SFR Cap Santé, UFR de Pharmacie, Université de Reims Champagne-Ardenne, 51 rue Cognacq-Jay, 51096 Reims, France.

² Sorbonne Université, Faculté de Médecine, Assistance Publique-Hôpitaux de Paris, Hôpital Pitié-Salpêtrière, Paris, France

*Corresponding author: reyol.cobalt@gmail.com

Keywords: taxonomy, molecular systematics, morphology.

Created inside the genus *Phlebotomus* as a subgenus then raised at the genus rank, up to now, the *Idiophlebotomus* includes 14 species all described from the oriento-australasian generalized track: *Idiophlebotomus asperulus*, *Id. pholetor*, *Id. stellae*, *Id. sejunctus*, *Id. erebicolus*, *Id. longiforceps*, *Id. frondifer*, *Id. tubifer*, *Id. teshi*, *Id. wellingsae*, *Id. dispar*, *Id. boucheti*, *Id. padillarum*, and *Id. nicolegerae*.

During the present study, we examined most of the type-specimens available and we also processed our own specimens using both morphological and molecular approaches (sequencing of D8 rDNA and COI mtDNA).

We did a critical analysis of the morphological characters used to identify the *Idiophlebotomus* species, with a particular focus on the spines and setae of the gonostyle commonly confused. Consequently, we tried to characterize them in order to provide a new identification key. We also updated the distribution areas of these species.

Molecular data support our morphological results and we finally discovered several new species to describe from the Philippines and Lao PDR.

A molecular systematics of the Psychodopygina (Diptera; Psychodidae) inferred from ribosomal and mitochondrial DNA

Zapata S^{*1,2}, Chaves JA³, Galati EAB⁴, Augot D², Le Pont F⁵, Gantier JC⁶, Bargues MD⁷, Mas-Coma S⁷, and Depaquit J²

¹Instituto de Microbiología, Universidad San Francisco de Quito, Quito, Ecuador

²Université de Reims Champagne-Ardenne, ANSES, SFR Cap Santé, EA 4688 – USC Transmission vectorielle et épidémiologie-surveillance de maladies parasitaires “VECPAR”, France

³Colegio de Ciencias Biológicas y Ambientales, Universidad San Francisco de Quito, Quito, Ecuador

⁴Departamento de Epidemiologia, Faculdade de Saúde Pública, Universidade de São Paulo, SP 01246-904, São Paulo, Brazil.

⁵72 Rue de la Colonie, Paris, France

⁶LIFE Lab., 4 Rue des semailles 91540 Mennecy, France

⁷Departamento de Parasitología, Facultad de Farmacia, Universidad de Valencia, Burjassot, Spain.

*Corresponding author: szapata@usfq.edu.ec

Keywords: Sandflies, rDNA, mtDNA, Bayesian analysis, phylogenetics

American sand flies are vectors of leishmaniasis, bartonellosis, and some arboviruses. In 1995, Galati proposed a new classification based on 88 morphological characters where the New World phlebotomines were organized in two tribes Hertigiini and Phlebotomini. The latter tribe structured in four subtribes (Brumptomiina, Sergentomyiina, Lutzomyiina and Psychodopygina) and 20 genera. The subtribe Psychodopygina comprised seven genera and included the majority of vectors of *Leishmania*. We tested Galati's proposal using combined sequences (1,334 bp) of two-genes: 28S rDNA (partial) and cytochrome *b* (mtDNA) from 47 taxa belonging to the Psychodopygina subtribe. Bayesian phylogenetic reconstruction confirms the monophyly of the genera *Psychodopygus* and *Psathyromyia*, whereas *Nyssomyia* and *Trichophoromyia* seem to be paraphyletic. The latter paraphyly is linked to the position of *Ny. richardwardi* which taxonomic position is doubtful. We were not able to determine whether the genera *Bichromomyia* and *Martinsmyia* were monophyletic because we analyzed only one species of each genus. Our molecular analysis provides additional support for the recent morphologic classification of the Psychodopygina subtribe.

Immune events triggered in host skin by a *Leishmania*-infected sand fly bite govern pathogenesis of leishmaniasis

Thiago DeSouza-Vieira¹, Eva Iniguez¹, Tiago Serafim¹, Ranadhir Dey², Subir Karmakar², Maria Disotuar¹, Waldionê de Castro¹, Tales Vicari Pascini³, Josh Lascina¹, Claudio Meneses¹, Valeria M. Borges⁴, Miguel Soares⁵, Nakhasi Hira², Fabiano Oliveira¹, Jesus G. Valenzuela¹ and Shaden Kamhawi*¹

¹Vector Molecular Biology Section, Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

²Laboratory of Emerging Pathogens, Division of Emerging and Transfusion Transmitted Diseases, Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, MD 20993, USA

³Molecular Parasitology and Entomology Unit, Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

⁴Gonçalo Moniz Institute, Oswaldo Cruz Foundation, Salvador, Brazil

⁵Instituto Gulbenkian de Ciência, Oeiras, Portugal

*Corresponding author: skamhawi@niaid.nih.gov

Transmission of *Leishmania* by bite of its sand fly vector involves several elements that contribute to parasite survival and disease establishment. Importantly, without these factors, leishmaniasis is significantly ameliorated, and in some instances abrogated. To date, our research and that of others focused on elucidating the mechanism by which vector-derived factors injected alongside parasites, such as saliva and the promastigote secretory gel, and more recently, exosomes and gut microbiota, augment the severity of leishmaniasis. Here, we present another facet to this complex event showing that *Leishmania* parasites also take advantage of an innate and conserved process, the wound healing response of its mammalian host. Female sand flies feed by lacerating capillaries in the skin to cause blood leakage from vessels. This leakage causes tissue resident macrophages and infiltrating monocytes to scavenge the escaped red blood cells leading to induction of the potent cytoprotective enzyme, heme-oxygenase 1 (HO-1). HO-1 is strongly induced 24-48h after sand fly bites and acts globally to reduce cytokine and chemokine levels and controls the high burst of IL1- β resulting from egestion of gut microbiota into the wound at the bite site. In the absence of HO-1, disease pathology is enhanced without a strong effect on parasite burden. Therefore, by dampening inflammation, HO-1 reduces disease pathology and promotes host tolerance to leishmaniasis that ultimately benefits the parasites. As such, we identify HO-1 as a central player in orchestrating the initial host immune response that determines disease outcome following the bite of a *Leishmania*-infected sand fly.

Lutzomyia longipalpis salivary gland extract modulates *Leishmania enriettii* infection in *Cavia porcellus*

Rodrigo Pedro Soares*¹

¹Instituto René Rachou/Fundação Oswaldo Cruz

*Corresponding author: rsoares@minas.fiocruz.br

Keywords: *Lutzomyia longipalpis*; Salivary glands extract; *Leishmania enriettii*; host-parasite interaction.

Salivary glands of arthropod vectors contain a wide variety of pharmacological substances known to play determinant roles not only during the feeding process, but also for parasite establishment and infection. In this work, the role of salivary gland extract (SGE) from *Lutzomyia longipalpis* was studied during the course of infection of *Leishmania (Mundinia) enriettii* in guinea pigs *Cavia porcellus*. In this host, the parasite causes a self-healing benign lesion that lasts around 3 months. This species is included in the newly described subgenus *Mundinia* that includes several *Leishmania* species that have human and veterinary importance such as *Leishmania orientalis*, *Leishmania macropodum* and *Leishmania martiniquensis*. *Leishmania enriettii* (L88 strains) was first isolated in Brazil from the guinea pig *C. porcellus* in the 1940s by Muniz and Medina in 1946. The suspected vector of *L. enriettii* is *Lutzomyia monticule* although confirmation following the Killick-Kendrick criteria was not provided yet. Forty years later, another strain (Cobaia), was isolated in 1985 from guinea pigs in a neighboring region. All histopathological studies that have already been performed in this species did not use salivary gland extract (SGE) and parasites were inoculated using needle. Our main hypothesis is that SGE from *L. longipalpis* could differentially modulate the course of the lesion and macrophage differentiation caused by avirulent (Cobaia) and virulent (L88) *L. enriettii* strains. Salivary glands of *L. longipalpis* were dissected and stored at -80 °C. Glands were sonicated for disruption and mixed with 10⁵ stationary promastigotes of *L. enriettii* prior to inoculation in *C. porcellus*. The nasal region was infected using needles with two strains of *L. enriettii* (L88 and Cobaia) in the presence/absence of SGE and followed for 12 weeks. Some L88-infected animals could develop ulcerated/nodular lesions, whereas Cobaia strain developed non-ulcerated nodular lesions. Animals experimentally inoculated developed a protuberance and/or lesion after the 4th and 5th weeks of infection. Macroscopically, the size of lesion in L88-infected animals was smaller in the presence of SGE suggesting a modulation by SGE from *L. longipalpis*. Also, remarkable differences were detected microscopically in the presence of SGE for both strains. After the 6th and 7th weeks, L88-infected animals were heavily parasitized with an intense inflammatory profile bearing amastigotes and pro-inflammatory cells compared to those infected by Cobaia strain. Morphometry analysis revealed that L1 macrophages were abundant in the L88 infection, but not in the Cobaia infection. In the presence of SGE, an increased CD163 macrophage infiltrate by both strains was detected. Interestingly, this effect was more pronounced in Cobaia-infected animals. This study showed the effect of *L. longipalpis* SGE during the course of *L. enriettii* (strains L88 and Cobaia) infection and its role in modulating macrophage attraction to the lesion site. SGE decreased L1 macrophages and this may favor an escaping mechanism for L88 parasites. On the other hand, in the presence of SGE, an increase in CD163C cells during Cobaia infection may be important for its control. Although both strains healed at the end of the infection, the role of SGE from *L. longipalpis* was determinant for the kinetics of the immunopathological events in this dermatropic species.

Funding: Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, PPM-X 00102-16) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 305065/2016-5).

Attractiveness of golden hamster (*Mesocricetus auratus*) to *Lutzomyia longipalpis* (Diptera:Psychodidae) before and after infection with *Leishmania (Leishmania) amazonensis*

Flávia Benini da Rocha Silva^{1*}, Vicente Estevam Machado¹, Dennys Ghenry Samillan Ortiz², Danilo Ciccone Miguel², Mara Cristina Pinto¹

¹Departamento de Ciências Biológicas, Faculdade de Ciências Farmacêuticas, Câmpus Araraquara, UNESP/Brasil

²Departamento de Biologia Animal, Instituto de Biologia, UNICAMP/Brasil

*Corresponding author: flavia.benini@unesp.br

Keywords: chemical ecology, cutaneous leishmaniasis, infected host, blood fed, blood volume

Lutzomyia longipalpis is the main vector of *Leishmania infantum*, aetiological agent of visceral leishmaniasis in South America, and it is considered a permissive vector of *Leishmania amazonensis*, one of the aetiological agents of cutaneous leishmaniasis. For culicidae, several studies demonstrate that *Plasmodium*-infected hosts are more attractive to *Anopheles* spp. in comparison with uninfected hosts. With respect to sand flies, there are some evidences for increased attractiveness of *L. infantum*-infected hosts to *Lu. longipalpis*. Regarding cutaneous leishmaniasis, such influence of infection on the attractiveness of *Leishmania braziliensis*-infected hosts was not observed for *Nyssomyia neivai*. The aim of this study was to evaluate the possible influence of *L. amazonensis* infection on the attractiveness of golden hamsters to *Lu. longipalpis*. Additionally, we also determined the blood volume ingested per sandfly female on the animals in both conditions. Initially, we evaluated the attractiveness of uninfected hamsters (n=3) to *Lu. longipalpis* and around the fifth week post-infection, the attractiveness of the same animals was evaluated for a second time by the same method. In all tests, the hamsters were anaesthetized with ketamine 10% (120mg kg⁻¹) and xylazine 2% (5mg kg⁻¹) and the attractiveness of each hamster in both conditions (uninfected and post-infection) was measured indirectly by the number of blood fed females. For the infection, 50 µL of PBS containing 2x10⁶ stationary-phase promastigotes of *L. amazonensis* PH8 strain were injected subcutaneously into the left hind footpad of three hamsters. Right hind footpads were not inoculated as an uninfected individual control standard. For attractiveness evaluation, hamsters were anaesthetized, housed individually inside a Barraud cage with 50 different couples of sand flies each and kept in the cages for a period of 45-60 minutes. After each attractiveness test, the sand flies were individualized and macerated in microtubes containing 125 µL of deionized water. After homogenization, 100 µL from each microtube were transferred to a 96-well plate for absorbance measurement at 540 nm, which is the wavelength of maximum absorbance for haemoglobin. Individual absorbance values were applied to the linear equation to estimate the blood volume ingested by each sand fly female. Analyzing the number of blood fed females, we obtained a mean of 18.6 blood fed females for uninfected hamsters, with 16, 21 and 19 on each animal, and 42.6 for the tests carried out post-infection, with 40, 43 and 45 blood fed females in each hamster. This increase in the number of blood fed females post-infection was statistically significant (p=0.0003). For the blood volumes, we can observe certain variability among the insects that fed in the same animal and also among insects that fed in different animals, with a total mean of 0.82 (±0.04) µL of blood ingested per sand fly female for uninfected and 1.24 (±0.03) µL per sand fly for post-infection (p<0.0001). For uninfected hamster 1, 2 and 3, there was a mean of 1.12 (±0.06), 0.75 (±0.07) and 0.62 (±0.06) µL of blood ingested per sand fly female, respectively. On post-infection, the mean blood volume ingested per sand fly on each hamster was of 1.20 (±0.05), 1.31 (±0.06) and 1.22 (±0.03) µL. Only hamster 1 did not present statistical difference (p=0.3523). Despite these tests are preliminary and a larger number of hamsters should be evaluated, our results show a relevant trend of increasing in the number of blood fed females and on blood volume ingested by the female sand flies when the animals are infected with *L. amazonensis*.

Financial support: CNPq (142092/2018-5).

A hamster model of defective sand-fly transmission may explain the occurrence of canine *Leishmania* seroreactors without evidence of infection in endemic areas of zoonotic visceral leishmaniasis

Luigi Gradoni^{1*}, Gioia Bongiorno¹, Eleonora Fiorentino¹, Valentina Foglia Manzillo², Manuela Gizzarelli², Gaetano Oliva²

¹Unit of Vector-borne Diseases, Istituto Superiore di Sanità, Rome, Italy

²Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy

*Corresponding author: luigi.gradoni@iss.it

Keywords: *Phlebotomus perniciosus*; hamster; experimental infection; IFAT; nested PCR

From field longitudinal studies there is evidence that a proportion of “resistant” dogs exposed to sand fly bites in *L. infantum*-endemic settings never manifest evidence of infection - i.e. parasite demonstration by microscopy/culture and DNA amplification from target tissues - while presenting a transient elevation of low-titre specific antibodies. The strong refractoriness to infection of these dogs, defined as belonging to an “exposed” stage of canine leishmaniasis, might result from a particular immunogenetic background or from natural booster doses determined by events of defective *Leishmania* transmission by the vector. Indeed, several studies report that even in ‘*Leishmania* sp/specific vector’ pairs, experimental infections fail to reach 100% development to metacyclic stages in infected sand flies despite foregut parasite colonization. Therefore, sand fly bites followed by regurgitation of non-infective promastigotes might be happening in nature and elicit antileishmanial antibody responses. To test this hypothesis, we developed a *Phlebotomus perniciosus*/hamster model in which conditions were artificially set to develop non-infective promastigotes stages colonizing the sand fly’s foregut. Archived data from 5 longitudinal field studies on *Leishmania* infections detected in naïve beagles exposed to 2 consecutive sand fly seasons, were analyzed. Dogs had been periodically examined over 2 years by serology (IFAT cut-off: 1/40), nested-PCR and culture from target tissues. Artificial membrane-feeding infection of colonized *P. perniciosus* was performed using *L. infantum* from N₂-cryopreserved batches of semi-purified amastigotes derived from heavily-infected hamster’s spleen. The parasites were used immediately after thawing for 10 min at 37°C and diluted to obtain 10⁷ amastigotes/mL of rabbit blood. Previously, we observed that this protocol resulted in low rates of blood-fed flies developing foregut infection, with negligible maturation to metacyclic forms. Naïve hamsters were exposed to the bite of artificially-infected sand flies which survived to take a second blood meal, and examined periodically for *Leishmania* over 9-11 months by the same assays used for dogs (IFAT cut-off: 1/10). Of 203 beagles exposed to the first sand fly season, 16% developed low (1/40-1/160) IFAT titres which maintained in some or declined to undetectable in other dogs. Despite having been exposed to a second season, none of these dogs showed evidence of *Leishmania* infection during the follow-up period, whereas subclinical or clinical infections were diagnosed in 48% of beagles. In eight experiments, 2818 sand flies were fed artificially with thawed amastigote-infected blood, of which 329 (12%) had a second blood meal on 40 hamsters. At the dissection performed immediately after the bites, 113 specimens (34%) showed foregut promastigote infections; none of the specimens which bit on 3 hamsters had anterior colonization of parasites, therefore these animals were excluded from further analyses. Only 1/37 hamsters developed full-blown leishmaniasis detected by all methods on month 10 p.i. Eleven hamsters (30%) showed low (1/10-1/80) and fluctuating IFAT titres mainly from 3 months after exposure, whilst both in vivo and post-mortem assessments for infection were found negative in these animals. Our model of defective sand-fly transmission can be helpful to explain the occurrence of healthy seropositive hosts in endemic settings of leishmaniasis.

Leishmania mexicana amastigotes of various origin: proteomic and developmental comparison

Lestinova T.^{*1}, Pacakova L.¹, Spitzova T.¹, Harant K.¹ and Volf P.¹

¹ Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic

*Corresponding author: terka.kratochvilova@seznam.cz

Keywords: *Leishmania mexicana*, amastigotes, sand fly, development, proteome

All *Leishmania* species share a digenetic life cycle characterized by motile promastigote stages that develop in the gut of phlebotomine sand flies and by non-motile amastigotes residing inside mononuclear phagocytic cells of vertebrate hosts. Thus, *Leishmania* parasites had to adapt to these two host environments that extremely differ mainly in pH and temperature. In natural conditions, amastigotes are permanently under the host's immune pressure and therefore they had to evolve strategies to avoid host innate immune responses, such as complement system, or intra-macrophage defense mechanisms like nitric oxide and reactive oxygen intermediates. In laboratory conditions, however, most studies on amastigote biology are done using *in vitro* macrophage infection systems (at vertebrate-reminiscent conditions) or using axenic amastigotes generated from promastigotes in cell-free medium by decrease of pH and increase of temperature. However, under these conditions, the pressure of host's environment is incomplete or even missing and this fact could be reflected in a different regulation of gene expression and protein translation. Therefore, our main aim was to study i) to what extent are axenically or macrophage-differentiated amastigotes similar to amastigotes originating from an infected mouse and ii) if all types of aforementioned amastigotes are equally useful for studies on parasite-vector interactions. To answer the first question, we used proteomic approach with quantification based on isobaric labelling (TMT 10 plex label) to characterize and compare proteome of all three types of *Leishmania mexicana* amastigotes. In samples from lesions, the main obstacle was the excess of vertebrate proteins. We tried different methods such as using gradient of metrizamide, polycarbonate tubes or erythrocyte lysis buffer to reduce content of vertebrate proteins and enlarge *Leishmania*-originating fraction. To answer the second question, we compared development of amastigotes in the natural vector *Lutzomyia longipalpis*. Females of *Lu. longipalpis* were fed through a chick-skin membrane on complement-inactivated rabbit blood with 3×10^6 amastigotes from different sources. Infection rate and localization of *Leishmania* infection in the sand fly midgut were studied by light microscopy. Morphological forms were determined and compared from midgut smears sampled at 1, 2, 3, 4 and 8 days post blood-meal.

Antibody response to Toscana virus, sandfly fever Sicilian virus and *Phlebotomus perniciosus* saliva in cats naturally exposed to sand fly bites

André Pereira¹, Nazli Ayhan^{2,3}, José Manuel Cristóvão¹, Petr Volf⁴, Lenea Campino¹, Remi Charrel^{2,4}, Carla Maia^{*1}

¹Global Health and Tropical Medicine (GHMT), Instituto de Higiene e Medicina Tropical (IHMT), Universidade Nova de Lisboa (UNL), Lisboa, Portugal

² Unité des Virus Emergents (UVE: Aix Marseille Univ, IRD 190, INSERM 1207, IHU Méditerranée Infection), Marseille, France

³Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic

⁴ Emerging Pathogens Institute, University of Florida, Gainesville, Florida

*Corresponding author: carlamaia@ihmt.unl.pt

Keywords: Cat, *Leishmania infantum*, *Phlebotomus perniciosus* saliva, Sandfly fever Sicilian virus, Toscana virus

Leishmaniosis and phleboviruses are emerging threats to animal and public health. Feline leishmaniosis and *Leishmania infantum* infection in cats have been documented in Portugal, together with the detection of antibodies to Toscana virus (TOSV) and Sandfly fever Sicilian virus (SFSV). Data from dogs and humans living in endemic areas of leishmaniosis suggest the use of antibody response to sand fly salivary antigens as an epidemiological biomarker for monitoring vector exposure. The aim of this work was to evaluate a possible association between exposure to *P. perniciosus* saliva with TOSV and SFSV in cats from Portugal. Out of 366 cats tested, eighteen (4.9%) and 8 (2.2%) were seropositive for TOSV and SFSV, respectively. Three cats were co-infected with TOSV and SFSV. Cats presenting antibodies to SFSV had significantly higher risk to be positive for TOSV. Seven cats positive to SFSV and 10 positives to TOSV had also significant levels of IgG to *P. perniciosus* saliva. A significant association was observed between the presence of antibodies to *P. perniciosus* and SFSV. The presence of antibodies to sand fly-borne viruses in cats, together with the detection of antibodies to *P. perniciosus* saliva, indicate that these animals are frequently exposed to sand flies and transmitted pathogens. Cats seem to be good sentinels for assessing human exposure to TOSV and SFSV. The development and implementation of efficient prophylactic measures directed to cats with the aim of reducing the prevalence of sand fly-borne pathogens in endemic areas would be useful.

Funding: CM and AP have the support of the Portuguese Ministry of Education and Science (via Fundação para a Ciência e a Tecnologia, I.P.), through an Investigator Starting Grant IF/01302/2015 and a PhD grant (SFRH/BD/116516/2016), respectively.

Host preference of the sand fly *Phlebotomus perfiliewi* in northern Italy

Mattia Calzolari^{*1}, Giuseppe Romeo¹, Federica Bergamini¹, Gianluca Rugna¹, Michele Dottori¹,
Elena Carra¹

¹ Istituto Zooprofilattico della Lombardia e dell'Emilia Romagna "B. Ubertini", Brescia, Italy

*Corresponding author: mattia.calzolari@izsler.it

Keywords: Host preference, *Phlebotomus perfiliewi*, *Leishmania infantum*

The host preference is a primary factor in determining the vectorial capacity of an arthropod for a particular pathogen, and then in shaping the cycle of the pathogen in a particular area. While every sand fly species may have a particular host preference, these insects are mainly described as opportunistic feeder, which feed on available animals. With the aim to clarify their host preference, we tested engorged females of sand fly collected in the hilly area of the Bologna province (Emilia-Romagna region, Italy). This area is characterized by a recrudescence of human Leishmaniosis in last years, and by very relevant densities of the sand fly *Ph. perfiliewi*, while the species *Ph. perniciosus*, the main recognized *Leishmania* vector in Italy, is less abundant. Two models of attractive traps were utilized to collect the engorged females in 12 sites: attractive trap baited by carbon dioxide (CO₂), and CDC light traps (CDC). A total of 13,429 sand flies were sampled, of which 2,028 were morphologically identified at species level (1,999 *Ph. perfiliewi*, 28 *Ph. perniciosus*, 1 *Ph. papatasi*). A total of 7,859 non-engorged females were grouped in 187 pools (with a maximum of 50 specimens per pool) and submitted to a *Leishmania*-PCR, detecting 46 positive pools. The engorged females sampled were 188: 169 from CDC, and 19 from CO₂. While CO₂ collected 8,413 sand flies (average of 312 per sample) and CDC 5,013 (average 209 per sample), CDC collected more engorged females. This result was justified by the attractive source employed by the traps, since carbon dioxide attract host-seeking female, which already digested a possible blood meal. The 124 engorged females were tested in single for (a) identification of the species by amplification and sequencing of the mitochondrial cytochrome c oxidase I gene (COI), (b) detection of *Leishmania* spp. DNA by Real-Time PCR, (c) identification of the blood meal by amplification and sequencing of two different targets: 350bp segment of the mitochondrial cytochrome b gene (cyt-b), using the modified vertebrate-universal specific primers cytB1-F and cytB2-R, and the single copy mammal prepronociceptin (PNO) gene. The source of the blood meals was obtained for 74 insects, of these 13 fed on two hosts. Most blood meals derived from the roe deer (46) followed by human (19), hare (6), horse (5), cow (3), boar (1). All these insects were *Ph. perfiliewi*, except a *Ph. perniciosus*, which fed on the boar. Interestingly one engorged specimen, which fed on the roe deer, was also positive for *Leishmania* spp. by Real-Time PCR. Since the positive results came at a low Real-Time Ct value (indicating a high number of parasites in the insect), we hypothesized that the sand fly was really infected, rather than parasites were simply present in ingested blood. In this case, the insect likely contracted the infection from a previous blood meal. Our results support, as already reported, the opportunistic feeding behaviour of *Ph. perfiliewi*, which bites available hosts. Nevertheless, this sand fly seems to prefer large-size animals, since blood of small mammals, as rodents (which were supposed to be present at the collection sites), were not identified. Interestingly, no dogs were recorded among bitten animals; this result was justified by the low presence of these animals in surveyed sites, and suggested the existence of a sylvatic life cycle of the parasite in the study area. Obtained results highlighted the feeding preferences of sand flies in the monitored area, and can give relevant clues to better clarify the *Leishmania* cycle in this particular nosogeographical entity.

An overview of Brazilian sand flies: Almost a century of scientific data and how fast are we moving on to uncover which are the truly vectors?

Felipe Dutra Rêgo^{1*}, Gustavo Mayr de Lima Carvalho^{1†}, José Dilermando Andrade-Filho^{1, 2, †}

¹Grupo de Estudos em Leishmanioses, Instituto René Rachou, Fundação Oswaldo Cruz, Av. Augusto de Lima, 1715, Barro Preto, 30190-002 Belo Horizonte, MG, Brazil

²Centro de Referência Nacional e Internacional para Flebotomíneos (COLFLEB), Instituto René Rachou, Fundação Oswaldo Cruz, Av. Augusto de Lima, 1715, Barro Preto, 30190-002 Belo Horizonte, MG, Brazil; [†] Equal contribution

*Corresponding author: felipedutra04@hotmail.com

Keywords: Diptera, Phlebotominae, leishmaniasis, sand flies, vector

The blood-feeding females of phlebotomine sand flies (Diptera: Psychodidae: Phlebotominae) are usually considered to be natural vectors of protozoan *Leishmania* species (Euglenozoa: Trypanosomatidae), the causative agent of leishmaniasis, a neglected tropical disease. There are over 900 species of sand flies recorded in the world. However, there is no consensus about the truly vectors involved in both wild and peridomestic cycles. A complete review of data concerning the number of species, the techniques used for the detection, the location of the study and the correlation between parasite and vector is not yet available. Here, we present a systematic evaluation of the sand flies species associated in the transmission of *Leishmania* in Brazil since the first record at 1922. A total of 73 species belonging to 14 genera have been reported associated to *Leishmania* parasites: *Bichromomyia* (3), *Evandromyia* (12), *Lutzomyia* (9), *Martinsmyia* (1), *Micropygomyia* (6), *Migonemyia* (1), *Nyssomyia* (8), *Pintomyia* (6), *Pressatia* (1), *Psathyromyia* (5), *Psychodopygus* (15), *Scyopemyia* (2), *Trichophoromyia* (3) and *Viannamyia* (1). From 1922 until the use of molecular methods in the 90s-decade, 30 species have been associated to *Leishmania* parasites, due to midgut dissection and further confirmations such as monoclonal antibodies, isozyme analysis, hamster inocula and in vitro culture. In the last three decades especially due to PCR methods, 43 other species have been reported carrying *Leishmania*-DNA. The criteria for vector incrimination, even established and recently updated, need to be reviewed. Based on that, some authors suggest that only seven species of sand flies fit some criteria for vector incrimination: *Br. flaviscutellata*, *Br. olmeca*, *Lu. longipalpis*, *Ps. wellcomei*, *Ny. neivai*, *Ny. whitmani* and *Ny. umbratilis*. The limitations to confirm the truly vectors of *Leishmania* in Brazil hold on until today, indicating the need to move toward to researches that clarify the capacity and vectorial competence of Brazilian sand flies.

Ecology and biting behavior of *Phlebotomus pedifer* in a cutaneous leishmaniasis hotspot in southern Ethiopia

Myrthe Pareyn^{*1}, Abena Kochora², Emma Van den Bosch¹, Luca van Rooy¹, Nigatu Girma², Fekadu Massebo², Herwig Leirs¹

¹ Evolutionary Ecology Group, Department of Biology, University of Antwerp, Belgium

² Department of Biology, Arba Minch University, Ethiopia

*Corresponding author: myrthe.pareyn@uantwerpen.be

Keywords: *Phlebotomus pedifer*, ecology, bloodmeal preference, biting rhythms, Ethiopia

Ochollo is a village in the mid-highlands of southern Ethiopia where cutaneous leishmaniasis (CL) is endemic. Over the past decades, the high CL burden has attracted several researchers, who estimated the prevalence of active disease at around 4% and stated that lesions are mainly seen on young children's faces and extremities. Other studies reported *Phlebotomus pedifer* as the main vector for *Leishmania aethiopica* in Ochollo. Hyraxes, living in caves in close proximity to *P. pedifer*, were suggested to be the reservoirs of this zoonotic infection. Understanding the transmission dynamics of CL is a prerequisite for efficient disease control, which is why our study investigated the ecology and behavior of *P. pedifer*, one of the most important vectors of CL transmission in Ethiopia and Kenya. For the current study, sand flies were captured on a monthly basis in different habitats between March 2017 and August 2018. Records of temperature and humidity were taken during the sampling period. Sand flies were screened for the presence of kinetoplast DNA to estimate the prevalence of *Leishmania* infected sand flies. With these data, seasonal patterns and habitat preference of the (infected) *P. pedifer* population were assessed. The blood meal origin of engorged sand flies from different habitats was determined by amplifying and sequencing a fragment of the Cytochrome B (CytB) gene. Additionally, the host preference of *P. pedifer* was established in an experimental set-up, where field captured sand flies were collected in a middle cage, while a human and hyrax were exposed in bilateral cages. Sand flies were allowed to take a blood meal, which was later determined by CytB sequences. Human landing catches were carried out indoors and outdoors over night to determine at which time sand flies are mostly active and whether they are exo- or endophagic. This study revealed that the *P. pedifer* population was negatively correlated with humidity, hence the species was most abundant in the dry season. Infected sand flies were significantly more present in caves. Humans appeared to be the most important blood meal source, even in caves, where hyraxes are mainly living. The feeding experiment will reveal the blood meal preference of *P. pedifer* when both hosts are equally available. *P. pedifer* was biting outdoors as well as indoors, with an endophagic preference, and was mostly searching for a blood meal around midnight. Our research provides information for guidance of vector control programs in areas burdened with CL in Ethiopia and Kenya. Interventions on *P. pedifer* should be applied in the wet season, when the population is lowest. A combination of indoor and outdoor vector control measures is recommended. Outdoor transmission can be tackled by emphasizing control on caves, while people should be protected by impregnated bed nets to target sand flies indoors when they are mostly active.

Influence of paromomycin resistance on *Leishmania* fitness inside the sandfly vector

Sarah Hendrickx¹, Lieselotte Van Bockstal¹, Jovana Sadlova², Kamhawi Shaden³, Petr Volf²,
Caljon G.^{1*†}, Louis Maes^{1*†}

¹Laboratory for Parasitology, Microbiology and Hygiene, University of Antwerp, Antwerp, Belgium;

²Department of Parasitology, Faculty of Science Charles University, Prague, Czech Republic;

³Vector Molecular Biology Section, Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States; †Equally contributed senior authors

*Corresponding authors: Louis.Maes@uantwerpen.be and Guy.Caljon@uantwerpen.be

Keywords: Paromomycin, resistance, fitness, sand fly infection

Since miltefosine monotherapy against visceral leishmaniasis (VL) has been discontinued in the Indian subcontinent due to an increase in the number of treatment failures, single dose liposomal amphotericin B is now advocated as a treatment option of choice. Paromomycin-miltefosine combination therapy can be used as substitute first-line treatment in regions without cold-chain potential. Previous laboratory studies have demonstrated that paromomycin monotherapy fairly rapidly selects for resistance associated with an increased fitness. Although no fitness gain was observed between wild type (WT) and PMM-R promastigotes, intracellular growth and *in vivo* infection capacity were clearly increased in PMM-R amastigotes. Given this enhanced fitness in the mammalian host, this study aimed to extend these findings by assessing the overall fitness of PMM-R parasites in the sand fly vectors *Lutzomyia longipalpis* and *Phlebotomus perniciosus* (for *L. infantum*) or *P. argentipes* (for *L. donovani*).

Although *L. longipalpis* is recognized as a susceptible fly for various *Leishmania* species, the infection burdens, metacyclogenesis rate and colonization of the stomodeal valve were considerably higher in the natural vectors *P. perniciosus* and *P. argentipes*. Despite these intrinsic differences in parasite susceptibility between the sand fly species, none of the models demonstrated significant dissimilarities between WT and PMM-R parasites. The fitness gain linked to PMM-R is not observed in promastigotes. Despite their unaffected behavior in the vector, the enhanced virulence observed for PMM-R amastigotes in the mammalian hosts may nevertheless represent an advantage for transmission as this may lead to higher numbers of parasites acquired by bloodfeeding sandflies. The results of such sandfly transmission studies on hamsters infected with either WT or PMM-R parasites will be presented.

Miltefosine resistance in *Leishmania*: What do sand fly infections teach us?

Lieselotte Van Bockstal¹, Sarah Hendrickx¹, Jovana Sadlova², Kamhawi Shaden³, Petr Volf²,
Louis Maes^{1†*}, Caljon G.^{1†*}

¹Laboratory for Parasitology, Microbiology and Hygiene, University of Antwerp, Antwerp, Belgium

²Department of Parasitology, Faculty of Science Charles University, Prague, Czech Republic

³Vector Molecular Biology Section, Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States; †Equally contributed senior authors.

*Corresponding author: Guy.Caljon@uantwerpen.be and Louis.Maes@uantwerpen.be

Keywords: Miltefosine, resistance, sand fly infection

Drug resistance or phenotypic adaptations of *Leishmania* leading to less effective treatment outcomes pose a significant threat to control programs in disease endemic countries. Since the introduction of miltefosine (MIL) in the Kala Azar elimination program, treatment failure rates have been steadily increasing. As a result, MIL has been abandoned as first-line therapy for visceral leishmaniasis in the Indian subcontinent. MIL therapeutic failure is known to have a multifactorial origin involving drug, host and parasite parameters. Parasites can acquire stable MIL-resistance by single point mutations in the *MT* gene, encoding a miltefosine/aminophospholipid transporter. In addition, karyotypic changes such as aneuploidy are a flexible way for parasites to increase tolerance to the drug. Understanding the propagation of such parasite traits requires an integrated view that not only considers infections in the vertebrate host but also in the sand fly vector.

Our studies have assessed the phenotypic effects of repeated MIL exposure on parasite infection characteristics in rodents and sand flies. The impact of these changes on infectivity, stomodeal valve colonization and metacyclogenesis were assessed in the two sand fly vectors, *Phlebotomus perniciosus* and *Lutzomyia longipalpis*. Also, the impact of MIL on the sand fly infection was explored because our recent findings in rodents identified a drug dependency of resistant parasites. Stability of the acquired traits after sandfly passage were evaluated to obtain insights in the transmission dynamics of MIL tolerance/resistance.

Repeatedly MIL-exposed parasites were found to reduce drug accumulation which results in increased tolerance or full resistance when alterations result in a defective MIL transporter. *MT*-deficiency severely impacted the various sand fly infection parameters. Episomal reconstitution with the wildtype *MT* gene partially restored infectivity. A such, a defect in the *MT* protein was found not only to cause MIL resistance but also to compromise development in the sand fly vector. Subtler changes increasing MIL tolerance, including a decreased copy of chromosome 13 harboring the *MT* gene, had no significant impact on sand fly infectivity. In combination with the stability of the acquired traits following sandfly passage, these results increase our understanding of the rise of treatment failures while maintaining a very low prevalence of MIL-resistant clinical isolates.

Antibodies against sand fly saliva: markers of host exposure to sand fly vectors

Volf, P.*¹, Spitzova, T.¹, Willen L.A.A.¹.

¹ Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic

*Corresponding author: volf@cesnet.cz

Keywords: *Phlebotomus perniciosus*, salivary antigens, marker of exposure

Sand fly saliva is composed of a diverse group of molecules (proteins and peptides) which have antihemostatic, anti-inflammatory and immunomodulatory properties. In naive hosts, *Leishmania* parasites benefit from saliva-altered local immune reaction: saliva causes "enhancing effects", reflected by larger lesions and higher parasite numbers. In contrast, hosts repeatedly exposed to bites of uninfected sand flies or immunized by salivary proteins were usually protected against *Leishmania* infection. Hosts repeatedly bitten by sand flies also develop specific anti-saliva antibodies. Levels of anti-saliva IgG reflect the intensity of exposure to sand flies and thus can be used in epidemiological studies, e.g. to measure the effectiveness of vector-control campaigns or as a marker of risk for *Leishmania* transmission. Around the Mediterranean basin, the sand fly *Phlebotomus perniciosus* is the principle vector of *Leishmania infantum*, the causing agent of canine leishmaniasis. In order to allow rapid evaluation of vector control programs and estimate the exposure of dogs to this vector, we studied specific IgG response against *P. perniciosus* salivary proteins in experimentally and naturally exposed dogs. Using ELISA, a salivary Yellow-related protein SP03B has been shown as the best marker to estimate canine exposure to *P. perniciosus*. Since standard serological methods, like ELISA, are impractical and time-consuming in field conditions, we developed the rSP03B sero-strip, a immunochromatographic test (ICT), that can be applied to screen large cohorts of dogs for the presence of anti-*P. perniciosus* antibodies. This colloidal gold ICT was prepared first with the bacterially-expressed rSP03B protein, it was highly sensitive and specific, and shown to be a valid replacement for standard ELISA assays. Even though the predictive values of this ICT were already in the excellent range – with a sensitivity of 100% and a specificity of 87% – further efforts were undertaken to increase the specificity of the test. Recently, we have optimized the ICT by using the rSP03B expressed in the Human Embryonic Kidney cell line, which further increased the specificity of the test to 95%. Moreover, we assessed the detection accuracy of the rSP03B sero-strip in field conditions by screening canine populations exposed to various frequencies of sand fly bites in different areas in the Mediterranean basin and collected on different time points. In order to exclude unfavorable cross-reactions between the proposed rSP03B sero-strip and non-vector sand fly species together with other hematophagous arthropods present in the Mediterranean basin, we tested the cross-reactivity between salivary antigens of *P. perniciosus* (whole SGH and rSP03B protein) and sera of animals that were experimentally bitten by *Ixodes ricinus*, *Ctenocephalides felis*, *Culex molestus*, *Phlebotomus papatasi*, *P. sergenti* or *P. tobbi*. Furthermore, we have optimized it with whole canine blood which renders it suitable for use in field conditions. The rSP03B sero-strip is the first rapid test designed for medical entomology research and it can be employed during epidemiological studies of canine leishmaniasis in the Mediterranean area.

Repeated sand fly bites have effect on *Leishmania* infection in BALB/c mice

Barbora Vojtkova*¹, Jovana Sadlova¹, Tereza Lestinova¹, Tatiana Spitzova¹, Jan Votypka¹
and Petr Volf¹

¹Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic

*Corresponding author: barbora.vojtkova@natur.cuni.cz

Keywords: leishmaniasis, sand fly saliva, cutaneous lesions, enhancing effect

Sand fly saliva have immunomodulatory effects on *Leishmania* infections in hosts. Co-inoculation of *Leishmania* parasites with salivary gland lysates (SGL) or the recombinant salivary peptides has been shown to enhance *Leishmania* pathogenicity. On the other hand, preimmunization of hosts with SGL or their pre-exposure to uninfected sand fly bites provided significant protection against infection (reviewed by Lestinova et al. 2017, PLoS Negl Trop Dis: e0005600). However, only a single study has been concerned on the effect of saliva in hosts previously infected by *Leishmania*; it described increased transmissibility of *L. donovani* to vector after multiple exposures of host to sand fly bites (Valverde et al. 2017, J. Infect Dis:1285-1293).

The aim of our work was to study the effect of exposure of *L. major* infected BALB/c mice to repeated sand fly bites. Mice were infected with sand fly derived *Leishmania* (Sadlova et al. 2015, Parasites & Vectors: 158) and divided into two groups, one of them being repeatedly exposed to *Phlebotomus duboscqi* females every two weeks and the second left as unexposed control. Course of infection was monitored weekly. The parasite load and distribution of parasites in various organs, particularly in the blood, skin and viscera of mice were tested *postmortem* using qPCR.

Repeated sand fly bites significantly affected cutaneous lesions. In mice exposed to sand flies, lesions developed faster and reached a larger size than in unexposed mice ($P = 0,048$). Multiple sand fly bites also increased parasites load in inoculated ears ($P = 0,085$). On the other hand, distribution of parasites in mice body and their infectiousness to vectors did not differ significantly between the experimental groups. These results suggest that multiple and repeated exposures of infected BALB/c mice to sand fly bites enhances the local skin infection caused by *Leishmania major* but does not affect the visceralization of parasites.

Acknowledgements: This study was funded by Czech Science Foundation GACR (grant number 17-01911S), GA UK (grant number 288217) and ERD Funds, project CePaViP (CZ.02.1.01/0.0/0.0/16_019/0000759).

Visceral leishmaniasis transmission in India: The role of xenomonitoring in a post-elimination setting

Shannon McIntyre^{*1}, Giorgia Dalla Libera Marchiori¹, Matthew Rogers¹, Vijay Kumar¹, Pradeep Das¹, Mary Cameron¹

¹London School of Hygiene & Tropical Medicine, Keppel St, London, WC1E 7HT, UK

*Corresponding author: shannon.mcintyre1@lshtm.ac.uk

Keywords: visceral leishmaniasis, xenomonitoring, *Phlebotomus argentipes*

In 2005, the governments of India, Bangladesh and Nepal signed a memorandum of understanding towards the elimination of visceral leishmaniasis (VL) as a public health problem, defined as less than one case per 10,000 people at block level administration. While this epidemiological target is a useful political tool, there is no evidence to suggest that achieving it will prevent future VL transmission and resurgence. In order to both achieve the elimination target and progress to breaking transmission, an improved understanding of VL transmission dynamics is required. Xenomonitoring involves measuring parasite DNA or RNA levels in the vector population, rather than the host. To date it has not been utilised in the surveillance of any *Leishmania* species. To assess the feasibility, scalability and cost effectiveness of integrating xenomonitoring into India's existing VL surveillance framework, and develop transmission assessment survey guidelines, a pilot study is currently in progress in the VL-endemic State of Bihar, India. CDC light traps are being used to sample *Phlebotomus argentipes* indoors in 120 households spanning three transmission strata: non-endemic, meso-endemic and endemic. Trapping will take place continuously throughout a 12-month period to ensure seasonal fluctuations in infection prevalence are captured in the dataset. PCR analyses will be carried out to detect all forms of *L. donovani*, and metacyclic forms specifically. Prevalence of infection and infectiousness will be calculated and compared with contemporary incidence data in humans to determine *P. argentipes* infectiousness prevalence required for transmission to human populations to occur. Recruitment of 120 households across 12 districts in Bihar was completed, and baseline questionnaires were conducted to assess household composition and characteristics associated with increased risk of *L. donovani* infection. Sampling of *P. argentipes* commenced in May 2019, and will continue for 12 months. Interim results will be presented. Integrating xenomonitoring into India's existing passive surveillance system could potentially help to overcome sensitivity constraints in traditional passive case detection, and indicate whether transmission within a given region is ongoing to assist in the deployment of VL control methods. Additionally, xenomonitoring has the potential to shed light on unknown aspects of VL transmission, including the entomological inoculation rate, natural rates of *P. argentipes* infection and infectiousness, and the role of asymptomatics and PKDL patients in maintaining transmission in the community. This information could be utilised to formulate transmission assessment surveys similar to those in use for lymphatic filariasis, to determine when transmission has been broken.

Are *Arvicanthis* and *Mastomys* Sub-Saharan reservoirs for *Leishmania major* and *L. donovani*? Xenodiagnoses by *P. duboscqi* and *P. orientalis*.

Jovana Sadlova*¹, Barbora Vojtkova¹, Katerina Hrnčirova¹, Tereza Lestínová¹, Tatiana Spitzová¹,
Tomas Becvar¹, Jan Votypka¹, Paul Bates² and Petr Volf¹

¹Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic

²School of Health and Medicine, Division of Biomedical and Life Sciences, Lancaster University, Lancaster, United Kingdom

*Corresponding author: sadlovaj@natur.cuni.cz

Keywords: leishmaniasis, wild reservoir, xenodiagnosis, Grass Rats, Multimammate Mice

Cutaneous leishmaniasis caused by *L. major* is a typical zoonosis circulating in rodents. In Sub-Saharan Africa, reservoir species remain to be identified although PCR positive mammals of various species were found repeatedly and *L. major* has been isolated from several rodents, including *Arvicanthis* and *Mastomys* spp. Visceral leishmaniasis caused by *L. donovani* is supposed to be mostly anthroponotic; a role for animal reservoir hosts has been suggested for years but not conclusively proven. Finding PCR positive animals does not necessarily mean they serve as parasite reservoirs infective to sand flies. Identification of reservoir hosts warrants longitudinal in-depth studies both in the field and in the laboratory, the best method for testing the infectiousness of hosts to biting vectors being xenodiagnosis. Here we studied experimental infections of *L. major* and *L. donovani* in *A. neumanni*, *A. niloticus* and *M. natalensis*; the infections being initiated either with sand fly-derived *Leishmania* or with culture-derived promastigotes. Inoculated rodents were monitored for several months and tested by xenodiagnoses for their infectiousness to *Phlebotomus duboscqi* and *P. orientalis*, natural vectors of *L. major* and *L. donovani* in Sub-Saharan Africa, respectively. The distribution and load of parasites were determined post mortem using qPCR from the blood, skin and viscera samples. In addition, host choice experiments with *P. duboscqi* and *P. papatasi* females were done. Three different *L. major* strains used significantly differed in infectivity: The Israeli strain (FVI) infected low proportion of rodents while two Sub-Saharan isolates from Senegal (LV109, LV110) infected high percentage of animals and produced higher parasite load in all the three rodent species. Infected animals showed temporary swellings and changes of pigmentation on the site of inoculation (ear pinnae). All three rodent species maintained parasites of the LV109 strain for 20-25 weeks and were able to infect *P. duboscqi*. However, infectiousness period was restricted to 5-10 weeks post infection in both *Arvicanthis* species and percentage of infected sand flies was generally low (0-10 %). In *M. natalensis*, parasites disseminated into different skin regions, the infection rates were high and the infectiousness period to sand flies long which make this rodent species promising reservoir in Sub-Saharan CL foci. On the other hand, none of animals inoculated with *L. donovani* were infectious to *P. orientalis* females although, in a part of animals, parasites were detected by PCR even 30 weeks post infection. Therefore, previous field findings of *Arvicanthis* and *Mastomys* infected with *L. donovani* should be evaluated rather as parasite sinks. This is indirectly supported also with results of host choice experiment which proved a high feeding rate of *P. duboscqi* on all tested rodents (40.5 – 93.5 %) while *P. orientalis* females significantly preferred man over both *Arvicanthis* and *Mastomys* and their feeding rate on rodents ranged between 1.4 and 5.8% only. Therefore, involvement of rodents in transmission of *L. donovani* by *P. orientalis* is very unlikely. Vector

Acknowledgements: This study was funded by Czech Science Foundation GACR (grant number 17-01911S) and ERD Funds, project CePaViP (CZ.02.1.01/0.0/0.0/16_019/0000759).

Leishmania infantum chagasi gene expression journey inside *Lutzomyia longipalpis*, the main vector of visceral leishmaniasis in Brazil

Thais Lemos da Silva¹; Erich Loza Telleria^{1,2}; Petr Volf²; Yara Maria Traub-Csekö^{1*}.

1. Instituto Oswaldo Cruz-Fiocruz, Rio de Janeiro - RJ - Brasil

2. Parasitology Department, Faculty of Science, Charles University, Prague, Czechia.

*Corresponding author: ytraub@ioc.fiocruz.br

Keywords: *Leishmania infantum chagasi*, *Lutzomyia longipalpis*, interaction, gene expression, CRISPR-CAS9.

We investigated *L. i. chagasi* genes that may play a role in the parasite-vector interaction. We performed qPCR to study *Leishmania* genes expressed during promastigote initiated infection in *L. longipalpis*. Among these are genes involved with sugar and amino acids metabolism, such as glucose transporters (GTs) and amino acid permease 3(AAP3). Both are more expressed at 1h, 6h and 72h post infection (PI) when there is higher parasite proliferation inside the insect. Polyubiquitin, which has a role in regulation of proteins important for parasite growth and differentiation, has higher expression at 144h PI, when large morphological changes of the metacyclic forms take place. Other genes studied were small endoplasmic reticulum-associated hydrophilic protein (SHERP), that is important in metacyclogenesis, and glycoprotein 63 (GP63), with a role in invasion of macrophages. Both have higher expression in late stages of insect infection, at 72h, 144h and 168h PI, indicating that while still inside the insect the parasite prepares itself for the contact with the vertebrate host. Elongation-1-alpha (EF1-alpha) is an important vertebrate host immunomodulator and is more expressed in the initial hours of infection in the insect, indicating that this gene may be involved in the initial establishment of infection in the vector. Xanthine phosphoribosyltransferase (XPRT), which is important in the purine salvage pathway, has reduced expression at 168h PI. Purines are relevant for vital cellular and metabolic processes such as proliferation. Nevertheless, in late infection it is possible that the parasite decreases purine acquisition due to the fact that metacyclic forms do not replicate. Furthermore, we investigated the role of the insect microbiota in the expression of parasite genes. Preliminary results obtained from infected insects treated with antibiotics, showed altered expression of some parasite genes. GTs expression decreased in early infection times and increased in late times in the absence of microbiota. Reduced bacteria in the midgut may expose the parasites to higher sugar concentrations, so an increased uptake of glucose to balance the osmolarity might be necessary. No difference was detected in expression levels of SHERP, GP63 and polyubiquitin during the course of infection in the absence of microbiota. We are presently investigating the metacyclogenesis process in the insects devoid of microbiota. Furthermore, we produced an *L. i. chagasi* EF1-alpha knockdown (KD) through CRISPR-CAS9 technique. These KD *Leishmania* in culture showed slower growth compared to wild type parasites. Artificial infection with *L. i. chagasi* EF1-alpha-KD indicated a loss of tropism in migration in the sandfly midgut since developmental forms of the parasite were not found in expected sites of the vector. Our findings show *Leishmania* genes modulated during the establishment of the infection, suggest that the gene expression of the parasite can be affected by the absence of bacteria and that EF1-alpha may have a sensory role for *Leishmania* inside the vector.

How to make a perfect cutaneous leishmaniasis outbreak in the Americas

Salomón OD* ^{1,2}.

¹Instituto Nacional de Medicina Tropical (INMeT), ANLIS “Dr. Carlos G Malbrán”, Puerto Iguazú, Misiones, Argentina.

²Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

*Corresponding author: odanielsalomon@gmail.com

Keywords: eco-epidemiology, edge effect, social determination

Outbreaks of American Cutaneous Leishmaniasis (ACL) are triggered by the confluence of multidimensional biological, climate, and social factors: the eco-epidemiological momentum. Despite the diversity of epidemiological scenarios, there are common "ingredients for the recipe" to make a perfect ACL outbreak. Describing the "where", "when", and "why" of this eco-epidemiological momentum could contribute to understanding the causes of epidemics, preventing their occurrence, and to defining better strategies to control them. Typically, due to sylvatic and rural transmission, most of the urban ACL cases are still related in time and space to peripheral deforestations, riparian forest, and green patches within the cities. Therefore, the "where" of the ACL outbreaks could be characterized in the space as an edge effect, and afterwards the "when" of this physical edge could be categorized in time as an ephemeral, transient or permanent edge. The "why" question is far more complex, as it includes the exposure of humans to vector due to anthropic activities in each kind of edge scenario. The evidence for ACL outbreak control strategies: barricading the edge by chemical and physical barriers, environmental management, and individual prevention, will be discussed. Answers to questions regarding these "where", "when", "why", and "how to control" guide the content of the questions to be asked in each new focus of ACL transmission, not only to mitigate transmission and prevent future outbreaks, but also to highlight the biological factors that might contribute to the possibility of an epidemic, and those conditions that modulate its actual probability, the climate and the social determination of risk.

Ecology of sand flies (Diptera: Psychodidae: Phlebotominae) in the municipality of Campo Grande, MS, Brazil

Wagner de Souza Fernandes*¹; Jucelei De Oliveira Moura Infran²; Samuel Lucas Lopes de Oliveira³; Aline Etelvina Casaril²; Elisa Teruya Oshiro²; Suellem Petilim Gomes Barrios¹; Everton Falcão de Oliveira¹; Alessandra Gutierrez de Oliveira^{1,2}.

¹ Fundação Universidade Federal de Mato Grosso do Sul, Faculdade de Medicina Drº. Hélio Mandetta, Programa de Pós-Graduação em Doenças Infecciosas e Parasitárias, Campo Grande, MS, Brasil.

² Fundação Universidade Federal de Mato Grosso do Sul, Instituto de Biologia, Campo Grande, MS, Brasil.

³ Fundação Universidade Federal de Mato Grosso do Sul, Faculdade de Ciências Farmacêuticas, Alimentos e Nutrição, Campo Grande, MS, Brasil.

*Corresponding author: wagner.ufms@gmail.com

Keywords: *Lutzomyia longipalpis*, spatiotemporal distribution, urbanization, visceral leishmaniasis

Brazil is responsible for 90% of visceral leishmaniasis (VL), a worldwide public health problem, in the Americas. Campo Grande, MS, Brazil, is an intense transmission area with the first case notified in 2001. The urban area of Campo Grande is growing which provides human contact with vegetation, favoring the circulation of parasites and vectors. The aim of this study was to identify the spatial and temporal distribution of sand flies and the risk-prone areas for the occurrence of VL in the city of Campo Grande. Sand flies were collected every fortnight using automatic light traps between July 2017 and June 2018 from 06:00p.m. to 06:00a.m. in 16 sites. Five of these sites were previously studied in 2000, 2004 and 2005 and the others were chosen according to the classification of "worst scenarios" for the occurrence of sand flies. The specimens were clarified and identified according to specific morphological key. A total of 384 collections were carried out, with a sampling effort of 4,608 hours. During this period 839 sand flies belonging to 4 species of different genus were collected: *Bichromomyia flaviscutellata*, *Evandromyia lenti*, *Lutzomyia longipalpis* and *Nyssomyia whitmani*. *Lu. longipalpis* was the most abundant species (n = 832/99.1%). Males were more frequent (M = 689 / F = 150), with a male-to-female ratio of 4.84. Highest density of sand flies was found in Moreninhas neighborhood (n = 245 / 29.4%), Tijuca (n = 148 / 17.8%) and Vivendas do Bosque (n = 121 / 14.5%). *Bichromomyia flaviscutellata* and *Ny. whitmani* were collected in the forest of Zé Pereira neighborhood. *Evandromyia lenti* was captured only in Monte Alegre neighborhood. There was an increase in the number of specimens between December and April, with peaks in January and March, coinciding with rainy periods in the state. When comparing the previously study in the municipality, we observed a drastic reduction in the composition and diversity of species (28 species in 1999-2000, 20 species in 2004-2005 and 4 species in 2017-2018). The municipality of Campo Grande is an endemic area to VL since 2002 and the presence of the main vector throughout the urban area demonstrates how the species is dominant and adapted to the environment, reinforcing the necessity for control measures by the competent organs.

Diversity, species composition and natural infection of sand flies vectors of cutaneous leishmaniasis along an altitudinal gradient on the Northwestern slopes of the Ecuadorian Andes.

Sandra Enríquez^{1*}, Jazzmín Arrivillaga-Henriquez², Alon Warburg³, Franklin Vaca¹, Vanessa Herrera¹, Washington Benítez-Ortiz¹

¹Instituto de Investigación en Salud Pública y Zoonosis, Unidad de Entomología Aplicada, Universidad Central del Ecuador. Quito, Ecuador;

²Facultad de Comunicación Social, Carrera de Turismo Histórico Cultural, Área de Ambiente, Universidad Central del Ecuador. Quito, Ecuador;

³Faculty of Medicine, Department of Microbiology and Molecular Genetics; Institute of Medical Research Israel-Canada; Kuvim Center for the Study of Infectious and Tropical Diseases, Hebrew University of Jerusalem, Jerusalem, Israel.

*Corresponding author: ienriquez@uce.edu.ec

Keywords: vectors, leishmaniasis, CDC light traps, natural infection, altitudinal gradient

The Northwestern of Pichincha province is an endemic area of cutaneous leishmaniasis, where anthropophilic sand flies with natural infection by *Leishmania* have been reported as vectors. Additionally, it is an area with a high diversity of flora and fauna because it is located in the Choco Biogeographic Region. However, this area has experienced considerable urban development in the last 40 years and many of its localities have been affected by the change in land use. The landscapes seen along the northwestern foothills of the Andes include pastures, cocoa, coffee and cassava crops and remnants of piedmont and cloud forests. We have conducted a research to know the diversity, species composition and natural infection of sand flies along an altitudinal gradient in the northwestern of Pichincha province. Sand flies were collected using CDC light traps in several localities in an altitudinal range of 250 to 1850 meters above sea level with presence or absence of cutaneous leishmaniasis. We also applied a CAP survey to determine the level of knowledge that people had about the disease. Male and female sand flies were separated and counted in the field. For laboratory identification, sand flies were dissected and mounted on glass microscope slides in Hoyer's medium. Sand flies were identified microscopically based on the cibarium, spermatheca and the external genitalia of males. *Leishmania* DNA in sand flies was detected by PCR amplification of the *Leishmania* specific ITS ribosomal DNA. Thirty-four species belonging to three genera were collected in the altitudinal gradient studied. The diversity and species composition changed along this altitudinal gradient, meaning that the greatest diversity was recorded at low altitudes and it decreased significantly at high altitudes. *Lutzomyia trapidoi* was a dominant species at low and intermediate altitudes but disappeared at high altitudes where it was replaced in dominance for other species. In addition, several sand flies were infected with some *Leishmania* species at low and intermediate altitudes, but this not occurred at high altitudes. This eco-epidemiological information agrees with the knowledge that people have about the disease along the altitudinal gradient studied. The disease was better known at low and intermediate altitudes where the main anthropophilic vector dominates, while it is totally unknown at high altitudes, where there are almost no vectors. These results are very important for understanding the dynamic transmission of cutaneous leishmaniasis (CL) in endemic areas.

Sand Fly Behavior: Much More Than Weak-Flying

Gabriel Barbosa Tonelli¹, Camila Binder Soares de Souza¹, Carina Margonari¹, José Dilermando Andrade Filho*¹

¹ Grupo de Estudos em Leishmanioses, Instituto René Rachou - FIOCRUZ

*Corresponding author: jose.andrade@fiocruz.br

Keywords: Phlebotominae, flying capacity, Psychodidae

Leishmaniasis is a disease transmitted by some species of sand flies and are widely distributed throughout the tropical areas. Despite the low mobility observed in the insect vectors of the disease, the geographical distribution of some species of sand flies is quite extensive, which hinders control and surveillance measures in endemic areas. The present study aims to continue the study of the ecology of Brazilian sand flies by analyzing the flying capacity of these insects, using for this the Velhas river that has approximately 180 meters of position between its banks. Four collections were carried out between 2017 and 2018, two in the dry season and two in the rainy season. Four HP-type light traps were arranged in the central area of the river, about 80 meters equidistant from each other, on an adapted stand, and also on the banks of the river. A trap was also installed on each bank of the Velhas river. The sample points were marked using GPS (Garmin®) and the wind speed was measured with anemometer. During the nights of collection, the wind speed was insignificant (<0.1 m / s). Six sand flies have been captured in the central region of the river, *Nyssomyia neivai*, *Ny. intermedia* and *Evandromyia lenti*. In relation to the margins, there were a total of 716 sandflies captured with greater abundance in margin 1 (71.65%). The most abundant species captured on the banks were *Ny. neivai* (64.8%) and *Ny. intermedia* (23.88%). The results obtained in the present work disagree with the data found in the literature regarding the flight capacity of the leishmaniasis vectors. Still, these findings directly infer the epidemiological complexity of the transmission of leishmaniasis in relation to the migration capacity of the vectors.

Bionomy of the phlebotomine sandflies vectors and ecoepidemiological modulation of leishmaniasis epidemic outbreaks in America, Africa, Europe and Asia

Iván Darío Vélez*¹

¹ Director PECET, Faculty of Medicine, University of Antioquia, Medellín-Colombia

*Corresponding author: ivan.velez@udea.edu.co

Keywords: Phlebotomine sandflies, bionomy, epidemic outbreaks

In endemic regions, the epidemiological risk of infection with *Leishmania* spp. is due to the presence, density and behavior of Phlebotomine sandflies vectors. Ecological factors, especially climatic but also social and cultural factors determine in certain circumstances the appearance of epidemic outbreaks of both cutaneous leishmaniasis and visceral leishmaniasis. The analysis of the transmission in endemic foci allows us to understand the fundamental role of eco epidemiology in the control of these diseases. For this science, vector insects are the fundamental element that explains the risks of transmission. The in-depth study of the bionomy of the vectors in a natural focus of infection allows us to develop rational, economic and effective prevention and control measures. The eco-epidemiology implies a holistic approach to the natural history of the disease inside the natural focus of infection. It determines the Phlebotominean species present in the focus, the identification of the vector species and their bionomy (spatial distribution, relative densities, sting habits and sting activity with respect to domiciles, seasonal variations of densities and the ecological factors related to the presence and density of the vectors). Eco-epidemiology also implies the study of parasite species and reservoirs; the clinical manifestations of leishmaniasis and human activities related to the greater risk of transmission; human activities that transform the environment affecting the modulation of the natural focus; clinical characteristics of the disease in humans and Conceptions - Attitudes - Beliefs and Practices regarding to the disease and the therapeutic responses. In this presentation, a description will be made of the epidemic outbreaks of cutaneous leishmaniasis caused by *Leishmania major* in the Maghreb region; the outbreaks of visceral leishmaniasis in immunocompetent patients and also of immunosuppressed in the Mediterranean basin, epidemic outbreaks of cutaneous and visceral leishmaniasis in the Americas and Asia and the recent epidemic outbreaks in sub-Saharan Africa.

Sandfly-borne phleboviruses circulating in Portugal: five and still counting

Fátima Amaro*¹, Líbia Zé-Zé¹, Maria Teresa Luz¹, Maria João Alves¹

¹ Centre for Vectors and Infectious Diseases Research, NIH Dr. Ricardo Jorge, Portugal

*Corresponding author: fatima.amaro@insa.min-saude.pt

Keywords: Phleboviruses, Portugal, Toscana virus, Arrabida virus, Alcube virus

In the Old World, sandfly-borne phleboviruses (genus *Phlebovirus*, family *Phenuiviridae*) are grouped into three species complexes: Naples, Sicilian (tentative species) and Salehabad. Sandfly fever Naples virus (SFNV) and Sandfly fever Sicilian virus (SFSV) are etiological agents of febrile syndromes. Adria virus, from the Salehabad (SALV) complex was associated with febrile seizure. Toscana virus (TOSV), which also belongs to the Naples species complex, can be responsible for central nervous system disease and is considered one of the most important causes of aseptic meningitis in summer months in the Mediterranean countries. In Portugal, the first reference to this virus was made in 1985, when its isolation from a tourist who became infected in Algarve, was reported in Sweden. Moreover, the first mention to SFSV presence in our country was made in 1974 and was regarding antibody detection in a seroprevalence study in humans. The objective of this work was to update the knowledge on the presence of sandfly-borne phleboviruses in Portugal. Clinical samples sent to the Centre for Vectors and Infectious Diseases Research (CEVDI/INSA) with a diagnostic request for arboviruses, or specifically for TOSV, were processed and tested by immunofluorescence assays and/or real-time RT-PCR. In addition, entomological surveys were conducted in the summers of 2007, 2008 and 2018, in Setúbal and Faro districts, in the south of Portugal, using CDC miniature light traps. Viral detection was performed by RT-PCR in sandfly pools, followed by sequence confirmation, isolation attempts and total genomic sequencing. Between 2009 and 2018, clinical diagnosis allowed the detection of 8 positive sera samples for TOSV, confirming the circulation of this virus in Portugal. Furthermore, in 2017 a pediatric case of SFSV infection was diagnosed. Regarding the entomological surveys, until now a total of 5325 sand flies organized in 415 pools (109 in 2007, 159 in 2008 and 147 in 2018) were studied. The isolation and complete sequencing of two new phleboviruses, Arrabida and Alcube viruses in Setúbal district (SFNV and SALV species complexes respectively), and the detection and complete sequencing of Massilia (SFNV species complex), in Setúbal and Faro districts was achieved. The species of sandfly from where these viruses were isolated was not determined but at least 10% of the sandflies collected at the same time and place were identified as *Phlebotomus perniciosus*, the most ubiquitous and abundant species in Portugal. Performed analysis to detect recombination events alerted for the possibility of Arrabida virus being a reassortant between Massilia/Granada and Punique-like viruses. Up to date, there are five confirmed phleboviruses circulating in Portugal: TOSV, SFSV, Arrabida, Alcube and Massilia. The first two are causing disease and were also found to circulate in domestic animals in seroneutralization studies, but the potential of Arrabida, Alcube and Massilia as pathogens is unclear and is under investigation. Our data indicates that at least, Arrabida, Alcube and Massilia viruses co-circulate in the same study area in Setúbal district while TOSV, Arrabida and Massilia co-circulate in Faro district. Preliminary sequence data also lead us to believe that other phleboviruses including new reassortant strains/varieties may be present. These results suggest that several phleboviruses and reassortants co-circulate in Portugal and are very likely transmitted by the same phlebotomine vectors.

This work was partially supported by an FCT project: Phleboviruses in Portugal: vectors, pathogenesis and co-infections (PTDC/DTP-SAP/0859/2014).

Updates on the distribution, diversity and abundance of sand flies (Diptera: Psychodidae) in Romania

Cristina Daniela Cazan*¹, Ioana Raluca Păstrav¹, Angela Monica Ionică¹, Gizem Oguz², Ozge Erisoz Kasap², Vit Dvorak³, Petr Halada⁴, Adriana Györke¹, Mirabela Oana Dumitrache¹, Petr Volf³, Bulent Alten², Andrei Daniel Mihalca¹

¹Department of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania

²Department of Biology, Ecology Section, Faculty of Science, VERG Laboratories, Hacettepe University, Ankara, Turkey

³Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic

⁴Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic

*Corresponding author: cristina.cazan@usamvcluj.ro

Keywords: sand flies, distribution, diversity, abundance, Romania

Phlebotomine sand flies (Diptera: Psychodidae) are haematophagous insects that transmit the protozoan parasite *Leishmania infantum* (Kinetoplastida: Trypanosomatidae), the main causative agent of zoonotic leishmaniasis in the Mediterranean basin. Eight sand fly species have been previously recorded in Romania: *Sergentomyia minuta*, *Phlebotomus papatasi*, *P. alexandri*, *P. sergenti*, *P. longiductus*, *P. balcanicus*, *P. perfiliewi*, and *P. neglectus*. The last three species are suspected or proven vectors of *L. infantum* in Balkan. Recent reports of autochthonous canine leishmaniasis cases present in the country require updates on sand fly distribution, diversity and abundance in Romania. In the current study, sand flies were collected in order to update these parameters in correlation with environmental and anthropic factors. Between 2013 and 2018, CDC Light Traps and mouth aspirators were used to collect sand flies in 132 locations from Romania, indoors and around various animal species shelters. In addition, one positive location (Fundătura village, Vaslui County, North-Eastern Romania) was selected for monthly collection (May-October 2017) to study seasonal dynamics. Animal-related and shelter-related data were recorded on site. For the second study, climatic parameters were collected (Romanian National Meteorological Administration) for each day of the sampling period and statistically correlated with the total number of collected sand flies and/or their presence (MedCalc software). Species identification was done using morphological keys, genetic tools and MALDI-TOF protein profiling. Sand flies were present in seven localities (5.3%): Eibenthal, Baia Nouă, Gura Văii (south-western Romania, Mehedinți County), Fundătura, Pâhnești, Epureni (eastern Romania, Vaslui County), and Schitu (southern Romania, Giurgiu County). Of the total number of collected sand flies (n=401), 209 (52.11%) were *Phlebotomus neglectus*, 189 (47.11%), *P. perfiliewi*, 1 (0.26%), *P. papatasi*, 1 (0.26%), *P. balcanicus*, and 1 (0.26%) *P. sergenti* sensu lato. We confirmed the presence of five sand fly species previously recorded in Romania, however, their updated distribution differs from historical data. The highest abundance was recorded at the beginning of August and the first day of capture was in mid-July. The diversity of sand fly species in Romania and their presence in areas with climatic influences constitutes a threat for the (re)emergence of vector-borne diseases. The statistical analysis of the climatic data showed a strong positive correlation between the total number of collected sand flies with the minimum and the maximum temperature and a negative correlation with the relative humidity. The analysis of the climatic data shows that the first presence of sand flies was registered only after the average minimum temperature for the previous 7 days was above 15°C. In the context of CanL and VL re-emergence in Romania, but also due to imported cases of the diseases in both humans and dogs, updates on vector distribution are needed.

Multi-scale influences on microbial sandfly community structure in Amazonia

A. Chavy^{1,2*}, A. Kocher^{3,4}, S. Tirera¹, M. Huguin¹, J-F. Guégan⁴, G. Prévot² & B. de Thoisy¹

¹Laboratoire des Interactions Virus-Hôtes, Institut Pasteur de la Guyane, Cayenne French Guiana

²Laboratoire des Ecosystèmes Amazoniens et Pathologie Tropicale, EA3593, Medicine Department, Université de Guyane, Cayenne, French Guiana

³CNRS, Université Toulouse III Paul Sabatier, IRD, UMR-5174 EDB (Laboratoire Évolution et Diversité Biologique), Toulouse, France

⁴Unité Mixte de Recherche MIVEGEC, Université de Montpellier, IRD, CNRS, 911 avenue Agropolis, 34394 Montpellier cedex 5, France

*Corresponding author: achavy@pasteur-cayenne.fr

Keywords: community, microbiome, ecology, sand fly, Amazonia

Research on the interactions of microbial vector communities with other pathogens has shown that bacterial communities can play a direct or indirect role in vector fitness and maintenance, survival, transmission and virulence of parasites. Studies on mammalian and plant models have shown that an imbalance caused by external disturbances of microbial communities could determine the patterns of transmission of infectious diseases. So far, most studies of bacteria in the midgut of sandflies focus on the possible use of these bacteria as a means of parasite or vector control. These bacteria, harmful or essential to vectors or parasites, can play a role in the transmission of leishmaniasis. However, no study has yet been devoted to the comparison of bacterial and sandflies communities and their respective structure. Here we expected to describe those communities, to characterize and quantify the relationships between the community networks of vectors and their microbial community. The analysis of vector and microbial communities coupled with a community ecology approach would also help to study what are the major environmental factors that drive and influence these communities. We conducted this study in French Guiana (South America) where the cutaneous leishmaniasis cycle is sylvatic and about 80 species of sandflies recorded.

We trapped sandflies 6 in forest sites using CDC Light Traps along a gradient of anthropogenic disturbances. For 4 sites there a gradient with 3 areas, one highly disturbed, one moderately disturbed and one slightly disturbed. Then, for each of these 3 points, we placed seven light traps along a 500 meter transects. This sampling allows studying the ecological factors structuring vector and microbiome communities at different scales. Relying on an anthropogenic gradient also allows analyzing the influence of the environment on these communities. Specific identification of sandflies and their microbiome were made by NGS using the marker for the 16S RNA ribosomal for the insect and the bacteria.

About 5,000 sandflies were collected from 206 communities. The 206 communities all together include 12 different genera. Preliminary results of the microbiome showed that the bacterial communities of both males and females were largely dominated by *Rickettsia*, *Wolbachia*, *Candidatus cardinium* and *Rickettsiella* bacteria.

The first results are encouraging because all those bacteria are known to have beneficial or deleterious effects on the fitness of arthropod vectors. Their presence may therefore have repercussions on the transmission of cutaneous leishmaniasis in French Guiana. Coupled with environmental data it will be interesting to see if the structure of these communities varies according to the different environmental scales and if they are explained by specific structures of sand fly communities.

Impact of anthropic activities on the diversity and distribution of sandflies species in the eastern region of Caldas, Colombia

Laura Posada-López*^{1, 2}, Jorge Alvar-Beltrán¹, Esteban Marín¹, Andrés Vélez-Mira¹ Iván Darío Vélez¹

¹ Programa de Estudio y Control de Enfermedades Tropicales - PECET, Universidad de Antioquia, Medellín, Colombia.

² Departamento de Epidemiologia, Faculdade de Saúde Pública, Universidade de São Paulo, SP 01246-904, São Paulo, Brazil.

*Corresponding author: lcposada@usp.br

Keywords: agroecosystems, phlebotomine, sand flies, Colombia

Anthropic activities lead to the loss of biodiversity and favors the dominance of some species; that often are better adapted and could acts as vectors of pathogens. About 63% of cases of cutaneous leishmaniasis have been reported in the eastern region of the Caldas state, in the last nine years. There, economy is based mainly on livestock, coffee, avocado, rubber and cocoa crops. However, the relationship between agricultural activities and the occurrence of CL cases has not been studied. Therefore, this study aimed to evaluate the impact of different agriculture activities on the diversity of sandflies in a region of the Caldas state. Data were collected in rural areas of Norcasia, Samaná and Victoria municipalities that are located in a range of altitude from 546 to 1228 meters a.s.l. We sampled the agroecosystems: forest, cocoa, coffee, avocado, rubber and pastures. To collect the sandflies, we used two CDC traps for each agroecosystem during two consecutive nights in November 2015, from 18:00 to 06:00 hours. The specimens were identified using the taxonomic key of Galati (2014). We estimated the diversity and Jaccard dissimilarity indexes to compare the agroecosystems and made cluster analyzes by place and by species of epidemiological importance using the free software R version 3.2.2 in vegan package. A total of 1242 sand flies belonging to 18 species were captured. The most abundant species was *Trichopygomyia triramula* (60%) and *Lutzomyia gomezi* (6%), both present in all agroecosystems. The higher diversity was observed in the forest (17 species), following the cacao (13 species) and rubber crops (12 species). The pasture (6 species) and coffee crops (4 species) showed lower diversity. The Jaccard dissimilarity analysis showed two different groups, one with high human intervention (avocado, coffee and pastures) and other with low intervention (forest, cocoa and rubber). In the high intervention group, it is important to remark the presence of Leishmania vectors such as *Lu. gomezi*, *Nyssomyia trapidoi* and *Ny. yuilli yuilli*. The sandflies diversity declined with the increase of human intervention in the crops, associated mainly to changes in the natural landscape and the use of the soil. This also is reflected in the higher dominance of some species that generally belong to leishmania vectors, such as *Lu. gomezi*. Our results suggest that anthropic activities affect the diversity and distribution of sandflies and could increases the risk of CL transmission. However, new studies including a long time series of sampling are necessary.

Phlebotomine sand flies and leishmaniasis in Oman

J.A. Rioux^{1†}, M. Gramiccia², N. Léger³, P. Desjeux⁴, & J. Depaquit^{*3}

¹ Faculté de Médecine, Université Montpellier 1, 1 rue École de médecine, 34000 Montpellier, France.

² Department of Infectious, Parasitic and Immune-Mediated Diseases, Unit of Vector-borne Diseases and International Health, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161, Rome, Italy.

³ EA 7510 ESCAPE - USC ANSES VECPAR, SFR Cap Santé, UFR de Pharmacie, Université de Reims Champagne-Ardenne, 51 rue Cognacq-Jay, 51096 Reims, France.

⁴ PATH OWH (formerly One World Health), A-9, Qutub Institutional area, USO Road, New Delhi, India.

†: in memoriam

*Corresponding author: jerome.depaquit@univ-reims.fr

Keywords: Molecular systematics, morphology, taxonomy, *Phlebotomus*, *Paraphlebotomus*, paleobiogeography.

Leishmaniasis remains poorly documented in Oman. A few case reports are available in the literature of both visceral and cutaneous leishmaniasis. A few studies have been carried out to know the sandflies fauna of the Sultanate. The goal of this eco-epidemiological study was on the one hand to isolate and to cultivate *Leishmania* strains in order to identify them from humans and animals and to inventory the Phlebotomine sandflies of the country in order to determine candidate(s) to the *Leishmania* transmission.

We prospected the two main biogeographical regions of Oman: Sharqiyah and Dhofar, the latter exhibiting phytogeographical originality (Afro-tropical elements, endemism).

We isolated and cultivated from patients two strains of *L. donovani*-MON31 in the Sharqiyah and one strain of *L. tropica* in the Dhofar. Regarding sandflies, we identified *Phlebotomus papatasi*, *Ph. bergeroti*, *Ph. duboscqi*, *Ph. alexandri*, *Ph. saevus*, *Ph. sergenti*, *Sergentomyia fallax*, *Se. baghdadis*, *Se. cincta*, *Se. christophersi*, *Se. clydei*, *Se. tiberiadis*, *Se. africana*, and *Grassomyia dreyfussi*.

Phlebotomus saevus is a candidate to the transmission of *L. tropica* in the Dhofar whereas the candidates to the transmission of *L. donovani* remain unknown in the Sharqiyah.

Diversity and ecology of phlebotomine sand flies (Diptera: Psychodidae) in the Quang Ninh province, a new update for Vietnam

Vu Sinh Nam¹, Tran Hai Son¹, Tran Vu Phong¹, Tran Cong Tu¹, Tran Nhu Duong¹, Dang Duc Anh¹, Nguyen Thi Yen¹, Vu Thi Lieu¹, Ngo Khanh Phuong¹, Nguyen Viet Hoang¹, Cécile Cassan², Nguyen Van Chau³, Anne Laure Bañuls²⁺ and Nil Rahola*²⁺

¹ National Institute of Hygiene and Epidemiology, Hanoi, Vietnam.

² UMR MIVEGEC IRD-CNRS-Université de Montpellier; Centre IRD de Montpellier, BP 64501, 34394 Montpellier, France

³ National Institute of Malariology Parasitology and Entomology, Hanoi –Vietnam

+ these two authors equally contributed.

*Corresponding author: Nil.Rahola@ird.fr

Keywords: Vietnam; Taxonomy; Inventory; South East Asia.

Several species of phlebotomine sand flies were collected during a study aiming to perform an update on the distribution and ecology of phlebotomine sand flies in the Quang Ninh province of Northern Vietnam where *Leishmania* cases were reported in 2001. We chose seventeen stations from three districts of the province with different ecosystems, from urban to rural areas. Phlebotomine sand flies were collected using CDC miniature light traps from May 30th to June 3rd 2016. A maximum of six light traps were set up in each site (inside and/or outside houses, animal barns, caves, crevices in the walls, etc.) and operated between 18:00 pm and 09:00 am during one night. A total of 68 light traps were set up and allowed us to collect 416 specimens (125 females and 283 males) amongst which 10 species of phlebotomine sand flies belonging to 4 genera were identified. The *Sergentomyia* genus was the most abundant (79.7% total), followed by *Phlebotomus* (13.7%), *Chinius* (6.1%), and *Idiophlebotomus* (0.8 %). We also found five specimens that we named *sp1* and which require further studies since they revealed unique characteristics and might constitute a new species. After a few entomological studies carried out in Vietnam (Raynal and Gaschen, 1934; Parrot and Clastrier, 1952; Quate, 1962; Lewis, 1978; Lewis, 1982; Lewis, 1987), the phlebotomine sand flies' fauna includes 11 species. Within the 10 species we collected, five were new species for Vietnam. This short study shows that Vietnam may be host to a much higher diversity of phlebotomine sand flies, therefore the whole country should be prospected in order to update the list of species and also their role in the transmission of *Leishmania* parasites and other infectious agents. Moreover, except for the species from South East Asia described by Raynal, all other species recorded in this area were described by Sinton from India even though the paleobiogeography of India is different from the one of South East Asia. There is also some surprising synonymizations that were proposed by both Lewis and Quate. For instance, *Se. hivernus*, which is a valid species, was curiously synonymized with *Se. iyengari*. Thus, at a larger scale, South East Asia needs an important revision of its phlebotomine sand fly fauna with description of new taxa, establishment of identification keys and characterization of *Leishmania* parasites and their vectors.

Altitude and hillside orientation as main factors of population structuring of *Phlebotomus ariasi*, vector of *Leishmania infantum*.

Jorian PRUDHOMME* §¹, Thierry DE MEEÛS^{§2}, Céline TOTY¹, Cécile CASSAN¹, Nil RAHOLA¹, Baptiste VERGNES¹, Bulent ALTEN³, Denis SERENO² and Anne-Laure BAÑULS¹.

1. MIVEGEC (IRD - CNRS - Univ Montpellier), centre IRD, Montpellier, France.
2. Intertryp, IRD, Cirad, Univ Montpellier, Montpellier, France.
3. Hacettepe University, Faculty of Science, Department of Biology, Ecology Section, ESRL Laboratories, 0680 Beytepe, Ankara, Turkey.

§ Authors equally contributed to this work.

*Corresponding author: jorian.prudhomme@hotmail.fr

Keywords: Sand flies, *Phlebotomus ariasi*, Population genetics, Ecotypes, Microsatellites markers

Despite their role in the transmission of *Leishmania*, still little is known on the organization of sand fly populations within their environment. In this context, we investigated the population genetic structure of *P. ariasi*, the main vector of *Leishmania infantum* in the region of Montpellier (South of France), using microsatellite markers. 1253 *P. ariasi* specimens were captured using sticky traps in 17 sites in the North of Montpellier along a 14 kilometers transect in 2011 from May to October for which environmental data (Such as altitude, hillside) were collected. We analyzed genetic divergence based on 11 microsatellite loci.

From the selected microsatellite markers, five loci were removed because of stuttering artefacts, absence of polymorphism or non-neutral evolution. The remaining six loci used revealed a significant heterozygote deficit in subsamples. A multiple regression approach showed the high influence of altitude (>50%) and low influence of geographic distance (7%) on the genetic data. The significant isolation by elevation observed in our data suggested a population structure of *P. ariasi* organized in altitudinal ecotypes with low migration rate between them.

This organization has undoubtedly implications on the ecology of sand flies and transmission of pathogens. These data highlight the necessity to consider sand fly population at small scales to study the ecology of these insects and the impact on *Leishmania* transmission.

What we have learned from sandfly-borne Phlebovirus researches in old world?

Nazli Ayhan¹, Remi Charrel^{1,2}

¹Unite des Virus Emergents (Aix-Marseille Univ – IRD 190 – Inserm 1207 – IHU Mediterranee Infection), Marseille, France.

²Emerging Pathogens Institute, University of Florida, Gainesville, Florida, USA.

*Corresponding author: nazliayhann@gmail.com

Keywords: Phlebovirus, sandfly-borne viruses, Sandfly fever Sicilian virus, Toscana virus

Phlebotomine sand flies are vector of several pathogen agents like bacteria (*Bartonella bacilliformis*), parasite (*Leishmania*) and viruses (*Phlebovirus*). Phleboviruses are public health treat and they show wide distribution in old-world. With this presentation, we aim to collect the phlebovirus identification, seroprevalence and epidemic/case report data from old-world in order to better understand phlebovirus characteristics with a comprehensive approach.

In last two decades, the number of the identified phleboviruses have been drastically increase both from clinical and environmental samples with correlated to increasing number of researches. From field collected sand flies, as a statistical phenomenon, higher sample size means high chance of phleboviral detection. Until now, co-circulation of phleboviruses have been showed in Turkey, Albania, France, Portugal, Italy and Tunisia with the variety in the number of naturally infected sand flies. In several studies, equal rates of female and male sand flies were identified as phlebovirus infected which suggest the transovarial (vertical) and/or venereal (horizontal) transmission of phleboviruses in sand flies. Contrary to initially believed, phleboviruses have been identified from different sand fly species which demonstrate that vectors are not highly virus specific in old world. Seroprevalence studies showed the high exposure to phleboviruses in both humans and animals in a wide geographic area in Old world from Mediterranean basin to North Africa and the Middle East to the central Asia.

Clinical studies introduced the epidemics of Sandfly fever Sicilian virus (SFSV) and Sandfly fever Naples virus (SFNV) in Serbia, Turkey, Greece, Cyprus, Egypt and Ethiopia with flu like symptoms. However, Toscana virus (TOSV) is the only sandfly-borne phlebovirus which cause central nervous system (CNS) infections with case reports from local people and visitors in endemic areas. TOSV is the major cause of infections like aseptic meningitis, encephalitis and meningo-encephalitis during warm season in endemic regions where its vector is present.

Continuing efforts to identify and characterize the phleboviruses are crucial to develop preparedness for possible epidemics.

Phlebotominae (Diptera: Psychodidae) and Biomes in the State of Mato Grosso do Sul, Brazil

S. P. G. Barrios¹, L. E. Pereira², A. E. Casaril¹, J. O. M. Infran³, W. S. Fernandes¹, E. T. Oshiro³, E. A. B. Galati⁴, G. Gracioli⁵, A. C. P. Filho⁶, A. G. Oliveira^{*1,3}.

¹ Programa de Pós-Graduação em Doenças Infecciosas e Parasitárias, Universidade Federal de Mato Grosso do Sul

² Programa de Pós-Graduação em Tecnologias Ambientais, Universidade Federal de Mato Grosso do Sul

³ Laboratório de Parasitologia Humana, Instituto de Biociências, Universidade Federal de Mato Grosso do Sul

⁴ Faculdade de Saúde Pública, Departamento de Epidemiologia, Universidade de São Paulo

⁵ Programa de Pós-Graduação em Ecologia e Conservação, Universidade Federal de Mato Grosso do Sul

⁶ Laboratório de Geotecnologias Ambientais, Universidade Federal de Mato Grosso do Sul

*Corresponding author: alessandra.oliveira@ufms.br

Keywords: Biodiversity, Inventory, Leishmaniasis, Pantanal, Sand flies.

The state of Mato Grosso do Sul, Brazil is an important region of South America regarding leishmaniasis, with great diversity of sand flies reported since 1938, and wide biodiversity represented by its biomes (Atlantic Forest, Cerrado and Pantanal). A checklist of sand fly (Diptera: Psychodidae: Phlebotominae) species is presented here for the state and respective biomes. The data base was compiled from primary and secondary data. The primary data collection involved automatic light traps at Miranda-Abobral Pantanal sub-region and in Baixa Nhecolância Pantanal sub-region. The secondary data were obtained from entomology scientific collections and a literature review of articles from 1938 to 2019, including entomological museum collections. A total of 71 species belonging to 15 genera and 13 subgenera were reported in 61 municipalities, including the type locality of nine species. Moreover, there was the first report of a *Leishmania infantum* (Nicolle, 1937) vector, *Lutzomyia cruzi* (Mangabeira, 1938), in Miranda-Abobral Pantanal region. Sand fly species incriminated as leishmaniasis vectors were reported in the entire area of the state, as *Bichromomyia flaviscutellata* (Mangabeira, 1942), *Lu. longipalpis* (Lutz & Neiva, 1912), *Nyssomyia antunesi* (Coutinho, 1939), *Ny. intermedia* (Lutz & Neiva, 1912), *Ny. neivai* (Pinto, 1926) and *Ny. whitmani* (Antunes & Coutinho, 1939). Other incriminated species were reported, such as *Lu. forattinii* (Galati, Rego, Nunes & Teruya, 1985), *Migonemyia migonei* (França, 1920), *Pintomyia fischeri* (Pinto, 1926), and *Pi. pessoai* (Coutinho & Barreto, 1940). A suspected species was also reported, *Lu. almerioi* (Galati & Nunes, 1999). Maps of the distribution of sand flies in biomes were designed. The species were considered eclectic to the three existing biomes.

Financial support: Fundect and Capes.

Sand Fly-borne Phleboviruses in the Balkan Countries

Nazli Ayhan¹, Remi Charrel^{1,2},

¹Unite des Virus Emergents (Aix-Marseille Univ – IRD 190 – Inserm 1207 – IHU Mediterranee Infection), Marseille, France.

²Emerging Pathogens Institute, University of Florida, Gainesville, Florida, USA.

*Corresponding author: nazliayhann@gmail.com

Keywords: Phlebovirus, sandfly-borne viruses, Sandfly fever Sicilian virus, Toscana virus

Phleboviruses are globally distributed agents which use ticks, mosquitos and sand flies as vector. Sand fly borne phleboviruses causing febrile diseases and central nervous system infections between April and October, depending on the region, correlated with vector sand fly activity. Balkan Peninsula is the first record of sand fly fever was originated and plays an important role as transboundary region between Asia and Europe. Novel phlebovirus identification and the activity of the phleboviruses have been reported from some Balkan countries, however, the knowledge is still limited with compared to other European countries. To be able to fill this gap, a comprehensive study was performed both containing identification of recognized and new phleboviruses from field collected sand flies.

A total of 6,352 sand fly individuals were collected from seven different Balkan countries (Albania, Bosnia-Herzegovina, Croatia, Kosovo, Montenegro, Macedonia and Serbia) in 2014, 2015 and 2016. Pools of sand flies (divided depending on sex, date and location up to 30 individuals) were tested for the presence of viral RNA, inoculated on VERO cell to be able to isolate the virus and specific real-time RT-PCR systems were designed from sequences corresponding to newly described viruses.

As a result, four novel phleboviruses (Balkan virus, Zaba virus, Bregalaka virus and Drin virus) belong to three different Phlebovirus serocomplex were detected and / or isolated. Balkan virus (within the Sandfly fever Naples serocomplex) was detected from *Phlebotomus neglectus* in Albania in 2014, one pool from Bosnia Herzegovina and four pools from Croatia in 2015. Zaba and Bregalaka viruses belong to Salehabad Virus group were isolated from sandflies from Croatia and Republic of Macedonia. Recently, Drin virus close to sandfly fever Sicilian viruses was identified from Albania. The full genome sequencing was performed for two isolated novel viruses with using NGS Ion Torrent PGM platform. Co-circulation of two lineages (Lineage B and C) of TOSV was detected in Croatia.

Our results show a variety of phleboviruses which belong to different genetic groups are co-circulating in the region. Based on our studies, Balkan area is one of the hot spots for phleboviruses in Mediterranean basin.

Field evaluation of transfluthrin treated military materials against Phlebotomine sand flies (Diptera: Psychodidae) in Thailand

Alongkot Ponlawat*¹, Nattaphol Pathawong¹, Arissara Pongsiri¹, Seth C. Britch², Robert L. Aldridge², Frances V. Golden², Silas A. Davidson¹, Kenneth J. Linthicum²

¹Department of Entomology, Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand

²USDA-ARS Center for Medical, Agricultural, & Veterinary Entomology, Gainesville, FL, USA

*Corresponding author: AlongkotP@afirms.org

Keywords: Transfluthrin, *Sergentomyia*, US military materials, spatial repellent.

The efficacy of transfluthrin applied on US military materials was field tested and we determined its impact on reducing sand fly populations in rubber tree plantations in Chanthaburi province, Thailand. Two different military barrier structures (HESCO; 2 x 2 x 2.5 m, and ULCAN; 3 x 3 x 2 m) lined with two different types of military materials including the thick felt-like material and the ultra-lightweight camouflage netting were constructed. The transfluthrin treated strips were hung inside the military barrier structures. The effectiveness of the transfluthrin treated strips was evaluated by sampling the natural sand fly population using CDC light traps baited with dry ice. Traps were operated for 14 hours (1800-0800) at 3 time points (1 day before application and 0, 2 weeks post application, respectively). All collected sand flies were separated by sex and morphologically identified to species level under the stereo microscopes and counted. The majority of sand fly samples captured in this study were *Sergentomyia iyengari*. The number of female sand flies collected by CDC light traps was analyzed using generalized linear mixed model with poisson distribution and log link function. The presence of transfluthrin treated strips significantly reduced sand fly populations up to 2 weeks post application. Our findings demonstrated that spatial repellent treatment of US military materials could potentially serve as an effective sand fly control in Thailand.

Attractiveness of a controlled release system with 1-hexanol to *Nyssomyia neivai* (Diptera: Psychodidae) in wind tunnel

Vicente Estevam Machado^{1*}, Flávia Benini da Rocha Silva¹, Mara Cristina Pinto¹

¹ Departamento de Ciências Biológicas, Faculdade de Ciências Farmacêuticas, Câmpus Araraquara, UNESP/Brasil

*Corresponding author: vicente.machado@unesp.br

Keywords: chemical ecology, controlled systems, 1-hexanol, wind tunnel (3-5)

Attractive baits are a possible tool to control and monitor hematophagous insects. Chemical ecology is the area which investigates these chemical compounds that can be used as bait. Previous studies in our laboratory showed that the compound 1-hexanol released in filter paper is a good attractant to *Nyssomyia neivai*, a suspected vector of *Leishmania braziliensis* in South America. An interesting approach in this field is the utilization of systems that modulated the release of chemical attractants for insects. Usually these release systems are developed with synthetic polymers, like polyethylene. However, in the pharmaceutical industry, natural polymers like gellan gum and pectin have been used as drug delivery systems because they are low-cost, nontoxic and stable. We developed two controlled release system using these polymers (Patent number: BR102019001965-4) which present physical differences in their structures. Both systems showed potential for modulating the release of the compound 1-hexanol. The aim of the present study was to evaluate the attractiveness to *Ny.neivai* of these controlled release system with 1-hexanol. The tests were carried out in a wind tunnel, with groups of three females placed inside releasing chambers. A total of 30 insects were used for each test. The releasing chamber is placed 50 cm from the chemical stimulus. We added 2 mL of the 1-hexanol in both controlled release system and evaluated them after 4, 24, 48, 60 and 72 hours. The tests lasted 2 minutes and we evaluate the activation (number of flies that leave the releasing chamber) and attraction (number of flies that reach the release system). For the control group we used the release system without any compound. The results for the group “1” were: 10% activated and 6.6% attracted. The responses of the sand flies for the evaluation at 4 hours were: 100% activated and 96.6% attracted, with a release rate of 105mg/h of the hexanol. After 24 hours 80% were activated and 53.3% were attracted, with a release rate of 132mg/h. After 48 hours 56.6% were activated and 30% were attracted, with a release rate of 65 mg/h. The sand flies were activated up to the 48h ($p < 0.001$) and at 60 to 72h the sand flies showed no response when compared with the control ($p > 0.001$). The sand flies were attracted up to 24h ($p < 0.001$) and showed no response in the other times compared to the control ($p > 0.001$). For the group “2”: 13.3% were activated and 3.33% were attracted. The responses of the sand flies for the evaluation at 4 hours were 96.6% activated and 93.3% attracted, with a release rate of 114mg/h of the hexanol. For the 24 hours 60% were activated and 43.3% were attracted, with a release rate of 90mg/h. The sand flies were activated and attracted up to 24h ($p < 0.001$) and at 48 to 72h the sand flies showed no response when compared to the control ($p > 0.001$). These results indicate that the release system “1” with hexanol can be more suitable to further experiments to get a better performance of the system and more studies in the field are necessary.

Financial support: CNPq (140298/2018-5).

Phlebotomus perniciosus biological adaptation under laboratory conditions

Gálvez R*¹, Macías Z¹, Sarquis J¹, Montoya A¹, Checa R¹, Marino V¹, Miró G¹.

¹Department of Animal Health, Veterinary Faculty, Universidad Complutense de Madrid, Spain

*Corresponding author: r.galvez@ucm.es

Keywords: *Phlebotomus*, sand fly colony, mass rearing, life cycle

Mass rearing of phlebotomine sand flies in the laboratory is considered a valuable tool that allows us to identify different biological mechanisms associated with these insects which is highly relevant in the Public Health. To learn about the duration of the different phases in the life cycle of these insects is crucial when applying appropriate control measures. The main objective of this study was to compare the reproductive success amongst wild phlebotomies (n=15; captured in 2017, first laboratory generation) and from colonized phlebotomies (n=60). The biological values analyzed were: a) Female mortality without oviposition; b) Mortality during the following 5 days after feeding; c) Average amount of eggs laid by females; d) Eggs viability (larval counting in relation to laid eggs); and, e) Productivity (average amount of adults born from each female that does the oviposition).

On individuals adapted to laboratory conditions, the different phases of the life cycle become shorter and the productivity increased. The number of females from the colony that did the oviposition is larger (Fisher test; p=0.0189). The preoviposition period (elapsed time between blood feeding and the lay of eggs) stretches on wild sand flies (Fisher test; p= 0.0028). The average amount of eggs laid by females from the colony was 42.4, significantly higher than the average from wild females, which was 26.3 (Wilcoxon test; p=0.0013). The productivity from the colony specimens was 7 adults per female, however the one from wild females was only 2 adults (Wilcoxon test, p= 0.0002). By contrast, there aren't differences in the egg's viability, causing an effective egg hatching in 50% of the cases (Chi-square test, p= 0.676).

In view of the results, those individuals that are best adapted to the laboratory conditions, are selected in a colony with time. This will allow the survival of the fittest, guaranteeing the successful continuation of the following generations.

A comparative study of three trapping methods for indoor sampling of *Phlebotomus argentipes* in the Indian subcontinent

Shannon McIntyre*¹, Giorgia Dalla Libera Marchiori¹, Matthew Rogers¹, Vijay Kumar¹, Pradeep Das¹, Mary Cameron¹

¹London School of Hygiene & Tropical Medicine, Keppel St, London, WC1E 7HT, UK

*Corresponding author: shannon.mcintyre1@lshtm.ac.uk

Keywords: vector sampling, surveillance, *Phlebotomus argentipes*

The number of visceral leishmaniasis (VL) cases in India, Bangladesh and Nepal has declined dramatically since a regional memorandum of understanding was signed in 2005, establishing an elimination target of <1 case/10,000 people at the block administrative level. As the VL epidemiological landscape continues to change, increased attention is being given to the need for surveillance of *Phlebotomus argentipes*, the vector of *Leishmania donovani* in the Indian subcontinent, to be routinely carried out, both to monitor the impact of control activities and to potentially identify pockets of ongoing transmission (xenomonitoring). The success of these surveillance activities is dependent on capturing large numbers of *P. argentipes*. Centres for Disease Control (CDC) light traps are widely used for sampling sandflies, including *P. argentipes*. In most published material to date, CDC light traps have only been able to collect small numbers of *P. argentipes*. To determine the optimal framework for mass-sampling of *P. argentipes* a field study was conducted in Bihar, India, comparing CDC light traps with two new suction-based techniques, namely Improved Prokopack aspirators (John Hock) and mechanical vacuum aspirators (Horst Armadihas). Three trapping methods, Improved Prokopack aspirators, mechanical vacuum aspirators and CDC light traps were selected for comparison. A total of 48 households (12 households/village, 2 villages/district, 2 districts, one VL endemic and one non-endemic) in Bihar were recruited for regular indoor sampling over a three-month period. Trapping was carried out four days per week, with the three methods rotated between each household based on a Latin Square distribution balanced for carryover effects. Samples were transported from field sites to Rajendra Memorial Research Institute in Patna, Bihar, for sorting and storage. Gel-based PCR following published protocols was used to speciate all sandfly samples identified as female through microscopy, and to identify blood meal source when necessary. The presence of all forms of *L. donovani* in samples identified as *P. argentipes* was assessed through PCR. Summary statistics and negative binomial regression were used to analyse all data. Sampling was carried out from June – September 2018, to give a total of 576 attempted trap nights. 13 trap nights were unsuccessful due to machinery failure and individual household complications. A total of 7156 (median 5, IQR 1-15) sandflies were captured during the sampling period, of which 3686 (51.5%) were identified as female. 1788 (48.5%) female sandflies were captured by CDC light traps, 1021 (27.7%) by Prokopacks, and 877 (23.8%) by mechanical vacuum aspirators. CDC light traps caught significantly more total sandflies ($p < 0.001$) and female sandflies ($p < 0.001$) than other methods, but were the least effective method for capturing blood fed female sandflies. A full cohort of speciated samples and analysis of infection status is forthcoming. This information will permit more nuanced recommendations on the appropriate technique for a xenomonitoring programme in India. Preliminary results suggest CDC light traps are the most effective method for capturing large numbers of sandflies, but not for trapping blood-fed female sandflies. Appropriate sampling methods for sandflies depend on the objectives of the study being performed.

Indoor residual spraying for the control of *Phlebotomus argentipes* in the Indian subcontinent: a review of the available evidence

Shannon McIntyre^{*1}, Graham Medley¹, Murari Lal Das¹, Mary Cameron¹

¹London School of Hygiene & Tropical Medicine, Keppel St, London, WC1E 7HT, UK

*Corresponding author: shannon.mcintyre1@lshtm.ac.uk

Keywords: Indoor residual spraying, *Phlebotomus argentipes*

Originally introduced in the 1950s as a malaria control method, indoor residual spraying (IRS) with Dichlorodiphenyltrichloroethane (DDT) was anecdotally credited for subsequent reductions in visceral leishmaniasis (VL) incidence observed in India, and adopted sporadically throughout the 1900s for VL management. In keeping with the recommendations of the World Health Organisation (WHO) Regional Technical Advisory Group (RTAG), IRS has been the mainstay control method for *Phlebotomus argentipes*, the primary vector of VL in the Indian subcontinent, since 2004. Following the replacement of DDT with alpha-cypermethrin in India in 2016, the governments of India, Bangladesh and Nepal now each carry out biannual application of synthetic pyrethroids in all areas considered to be endemic for VL.

Numerous observational and intervention trials have been conducted since the late 1990s to investigate the effectiveness of IRS under different insecticide regimes, however a consensus on its appropriateness as a control method for *P. argentipes* has not been reached. Understanding not only the effectiveness, but the acceptability, quality and cost efficiency of IRS in preventing VL transmission would allow the national programmes to make more informed decisions on the value of continuing spraying. For this reason, a systematic review of all available literature on IRS in the Indian subcontinent was carried out with the aim of assessing its current and future utility in VL control. ScienceDirect, PubMed and Web of Science, together with the grey literature databases opengrey.eu and WorldCat were searched up to February 2018. Additionally, the abstract booklets of WorldLeish and the American Society for Tropical Medicine and Hygiene (ASTMH) were searched for relevant unpublished material. Eligible studies were restricted to those carried out in India, Bangladesh and Nepal, and included primary data collection in the field. Studies reporting epidemiological outcomes (with diagnosis confirmed through parasitological or immunological recognised methods, such as rK39), entomological outcomes, quality, cost effectiveness, and acceptability were considered for inclusion. Standard data extraction forms following the framework of Munaro et al. (2007) were utilised. A total of 700 results were returned from all database searches. The titles and abstracts of each study were scanned for eligibility, and 47 papers progressed to full text screening; 19 studies met the eligibility criteria and were included in the final review. The selected studies evaluated four insecticides, DDT, alpha-cypermethrin, deltamethrin, and lambda-cyhalothrin, with DDT being the most common. Three studies presented results from randomised control trials (RCTs) evaluating entomological outcomes; no RCTs investigating epidemiological outcomes were identified. Only one study assessing cost effectiveness was available, four dealt with some aspects of quality, and four included qualitative accounts of acceptability. No high-quality studies supporting the continued use of IRS were identified.

The current evidence base does not support the continued use of IRS for VL control in the Indian subcontinent. There is an immediate need for RCTs primarily assessing the epidemiological impact of IRS. Alternative control methods, such as long-lasting insecticide treated nets, should be revisited for long-term control of VL in low transmission settings.

Bacterial infection and innate immune responses in sand fly larvae midgut

Marcelo Ramalho-Ortigao*¹ and Ju-Lin Weng Huang¹

¹ Department of Preventive Medicine and Biostatistics, F. Edward Hebert School of Medicine, Uniformed Services University, 4301 Jones Bridge Road, Bethesda MD 20814

*Corresponding author: jose-marcelo.ramalho-ortigao@usuhs.edu

Keywords: Sand fly larvae, bacterial challenge, innate immunity

Typically, insect innate immune responses including those in the midgut, result in the expression of effector molecules such as antimicrobial peptides (AMPs) that target bacterial, fungal and/or parasitic infections. In the insect midgut, these molecules are primarily governed by two signaling pathways, Toll and immune deficiency (IMD). While both pathways control systemic immune responses, AMP production in the intestinal epithelia depends predominantly on the IMD pathway whose activation occurs through peptidoglycan recognition proteins (PGRPs). Proper regulation and fine-tuning of insect innate responses like the IMD pathway and others such as DUOX (NADPH dual oxidase) in the gut are critical for insects. For the IMD pathway, knockout of *Relish*, a NF- κ B transcription factor, leads to an ineffective humoral response. Conversely, inappropriate triggering of the IMD pathway by activation of *Relish* leads to the over-expression of target genes that may be deleterious. The ubiquitous activation of the IMD pathway in *Drosophila* larval stages results in developmental abnormalities, whereas in adult flies the **chronic** activation of innate immunity pathways creates inflammatory-like conditions that significantly reduce the insect's lifespan. Using sand fly larvae, our results demonstrated that **acute** infection with the Gram negative enterobacterium *Pantoea agglomerans* led to extensive apoptosis at infection sites and up-regulation of transcripts associated with midgut epithelial cell proliferation and regeneration, whereas infections with Gram positive *Bacillus subtilis* led to a less severe response. Previous studies using gnotobiotic sand fly larvae revealed that certain bacteria negatively affect larval development, adult emergence, and survival of both larvae and adults. Hence, these results suggest a correlation of the larval innate responses to bacteria in the midgut, midgut dysbiosis, and the likelihood that prolonged exposure to certain bacteria may lead to an accentuated inflammatory state. All of which may account for insect survival and fitness. Thus, not only are different bacteria recognized by the distinct immune response pathways, but the ability to control these responses combined with epithelial regeneration to maintain gut homeostasis are critical for larval survival upon acute infection. Our results are also suggestive of the still uncharacterized cross talk that likely exists between the IMD and Toll pathways and likely involved in maintaining the homeostasis of the insect midgut. These and other aspects of the innate immunity of sand flies will be discussed within the scope of developing novel strategies for vector control and/or reduction of vectorial capacity of sand flies.

Zooprophylaxis: Impact of traditional breeding rabbits in man-made underground holes located in the peri-domestic areas on the transmission of zoonotic cutaneous leishmaniasis in Central Tunisia

Elyes Zhioua^{*1}, Ifhem Chelbi¹, Walid Barhoumi¹, Khalil Dachraoui¹, Talel Ben Slimane¹, Wasfi Fares¹, Wael Fraihi¹, Belhassen Kaabi¹, Richard S. Ostfeld²

¹ Pasteur Institute of Tunis, Tunis, Tunisia

² Institute of Ecosystems Studies, Millbrook, NY, USA

*Corresponding author: elyes.zhioua@gmail.com

Keywords: Zooprophylaxis, *Phlebotomus papatasi*, *Leishmania major*, Zoonotic cutaneous leishmaniasis

Zoonotic cutaneous leishmaniasis (ZCL) caused by *Leishmania major* and transmitted by *Phlebotomus papatasi* is endemic in Central and Southern Tunisia with an annual incidence rate of 670 cases per 100,000 inhabitants. Although ZCL is generally not fatal, the lesions produced may cause substantial disfigurement and severe distress to infected individuals with lifelong psychological and social consequences. No effective vaccine is available and treatment is based on chemotherapy with potential development of resistance by the parasite. Vector control is based on the use of chemical insecticides, which are expensive, and their applicability is limited by financial constraints in low-income countries in addition to its adverse environmental effect. We aim to develop a biological and sustainable method to reduce the transmission of ZCL in endemic areas. Zooprophylaxis is the use of animals to deviate vectors from humans. The indoor abundance of *P. papatasi* in houses with rabbit holes in the peridomestic areas is significantly lower than the indoor abundance in houses without rabbit holes in their peridomestic areas. Introduction of rabbits in artificial underground holes in peridomestic areas reduced significantly the indoor abundance of *P. papatasi* and the infection rates of sandflies with *Leishmania major*. Active rabbit holes made around houses in endemic areas is a major source of attractiveness of *P. papatasi* and therefore it may deviate the vector from humans to rabbits. While rabbit holes are breeding sites for *P. papatasi*, rabbits are not competent reservoir for *L. major* and subsequently, rabbits could be used for zooprophylaxis. Our overall findings strongly suggest that zooprophylaxis could be effective in reducing the indoor abundance of *P. papatasi* and their infection with *L. major* and subsequently may be used to control the transmission of *L. major* in endemic areas.

This project is funded by the US National Institutes of Health/National Institute of Allergy and Infectious Diseases: R21AI11710 (2016-2019).

Laboratory reassessment of the anti-feeding effect of Scalibor® deltamethrin collar against the sand fly *Phlebotomus perniciosus* in dogs confirms elevated and sustained efficacy for one year following treatment

Alec Evans¹, Gioia Bongiorno², Josephus J. Fourie³, Nouha Lekouch¹, Riccardo Bianchi², Cristina Khoury², Emmanuel Thomas⁴, Luigi Gradoni^{*2}

¹Clinvet SA Morocco, Mohammedia, Morocco

²Unit of Vector-borne Diseases, Istituto Superiore di Sanità, Rome, Italy

³Clinvet International (Pty) Ltd, Bloemfontein, South Africa; ⁴MSD Animal Health Innovation GmbH, Schwabenheim, Germany

*Corresponding author; email: luigi.gradoni@iss.it

Keywords: deltamethrin collar; *Phlebotomus perniciosus*; dog; anti-feeding efficacy; fast-killing efficacy

The objective of this parallel group designed, single centre, placebo controlled, randomized, blinded, efficacy study was to determine the anti-feeding and fast-killing efficacy of Scalibor® deltamethrin collars against experimental *Phlebotomus perniciosus* sand fly challenges on dogs, over a period of one year.

Twenty Beagles of both sexes aged ≥ 6 months were first evaluated for their attractiveness to sand flies for random allocation to the study groups. Suitability of dogs for inclusion was assessed using the proportion of blood-fed sand flies following exposure to 40-75 *P. perniciosus* females. Sixteen dogs were included in the study randomly allocated to two groups of 8 dogs each, fitted with placebo collars (control group) and deltamethrin collars (treated group) on Day 0, respectively, based on blood feeding rates and sex distribution used as ranking criteria. Specific health observations for acute adverse events were performed during 24 hours after collar administration. Daily veterinary care was ensured throughout the study period. For artificial sand fly challenges, sedated dogs were exposed for 60 minutes to approximately 95 (85 females, 10 males), 7-13 (+1) day-old unfed *P. perniciosus* sand flies in cages of 81 x 58 x 58 cm dimensions, on Days 28, 56, 84, 112, 140, 168, 197, 224, 253, 280, 308, 337 and 364. At the end of the exposure, all live (= able to fly normally) sand flies were collected from challenge cages into the same vented container using a battery-powered mechanical aspirator. Each dog was checked prior to and after removal from the cage to collect any visible flies, either live, moribund (= uncoordinated movements and unable to fly) or dead. Compressed air was blown into the dog's hair to help recover of any remaining insects. All dead sand flies were collected using forceps, and placed into a separate vented container. Live sand flies were immobilized using CO₂ or by freezing, prior to evaluating the engorgement status in females. Live, moribund and dead females were categorized as fed or unfed. Hence, the sand flies were assessed according to the following categories: live and unfed; live and fed; moribund and unfed; moribund and fed; dead and unfed; dead and fed. One dog from the treated group was excluded from the study after damaging its collar on Day 21. Another dog from the treated group was excluded from the Day 364 assessment, as it destroyed its collar. Dermatitis of the skin in the neck region was reported for 2 dogs of the treated group in the first two months of collar application. In both cases the conditions resolved with the aid of concomitant therapy. Whilst the fast-killing efficacy did not reach 80% over the challenge period, the anti-feeding efficacy rates exceeded 90% on all time points till Day 364, with the exception of Day 337 where it was 89.3%. At the last time point (Day 364), the anti-feeding was higher than 95%. Furthermore, the number of live sand flies, as well as the number of fed sand flies in the treated group, were statistically lower compared to the placebo control group for all challenge occasions. Scalibor® deltamethrin collar was regarded effective in preventing feeding of *P. perniciosus* for up to a one-year period.

Yellow-related proteins in saliva of *Phlebotomus orientalis*: amine-binding and antigenic properties

Sumova, P.*¹, Sima, M.¹, Spitzova, T.¹, Osman, M. E.², Guimaraes-Costa, A.³, Oliveira, F.³, Elnaiem, D. A.⁴, Hailu, A.⁵, Warburg, A.⁶, Valenzuela, J. G.³, Volf, P.¹

¹ Dept. of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic

² Commission for Biotechnology and Genetic Engineering, National Centre for Research, Khartoum, Sudan

³ Laboratory of Malaria and Vector Research, NIAID, National Institutes of Health, Rockville, Maryland, USA

⁴ Dept. of Natural Sciences, University of Maryland Eastern Shore, Princess Anne, Maryland, USA

⁵ Dept. Microbiol., Immunol. and Parasitol., College of Health Sciences, Addis Ababa University, Ethiopia

⁶ Dept. Microbiol. and Mol. Gen., The Kuvin Centre for the Study of Infect. and Trop. Dis., The Hebrew University of Jerusalem, Israel

*Corresponding author: sumovap@natur.cuni.cz

Keywords: *Phlebotomus orientalis*, yellow-related protein, biogenic amine, marker of exposure

Salivary yellow-related proteins (YRPs) form an abundant protein family reported in all sand fly species. However, their structure and amine-binding function were studied solely in *Lutzomyia longipalpis* YRP LJM11. Here we have focused on determining the function and antigenic properties of YRPs derived from *Phlebotomus orientalis*, a vector of *Leishmania donovani*, the causative agent of visceral leishmaniasis in Eastern Africa. The microscale thermophoresis method was used to determine the amine-binding interactions of two recombinant *P. orientalis* YRPs, rPorASP2 and rPorASP4, with serotonin, histamine and catecholamines. Low affinity binding was demonstrated for histamine, which was not bound tightly enough to prevent its interaction with physiological receptors. On the other hand, both rPorASP2 and rPorASP4 were demonstrated as high and medium affinity binders of serotonin, respectively; thus, proving their capability to facilitate inhibition of blood vessel contraction and blood clotting. This anti-hemostatic potential could be further enhanced by medium affinity binding of norepinephrine measured for rPorASP4. Using the *L. longipalpis* YRP LJM11, we have shown the comparability of the microscale thermophoresis method with the conventional isothermal titration calorimetry. By homology modeling, we have demonstrated the surface charge and both amino acids and hydrogen bonds of the amine-binding motif to influence the binding affinities of YRPs. The kratagonists function of YRPs in genus *Phlebotomus* was demonstrated for the first time. The YRPs were also repeatedly shown as major antigenic proteins eliciting specific anti-sand fly responses in bitten hosts. To identify valid markers of exposure to *P. orientalis* in humans, we have screened for anti-*P. orientalis* antibodies in sera of people residing in endemic areas of Sudan and Ethiopia, using either *P. orientalis* salivary gland homogenates (SGH) or individual recombinant salivary proteins as antigens. The rPorASP4 was identified as one of the most promising antigens showing high correlation coefficients as well as good specificity in comparison to the SGH, while the other YRP (rPorASP2) was not found antigenic. Combination of rPorASP4 and antigen 5-related protein led to a further increase of correlation coefficients as well as both positive and negative predictive values of *P. orientalis* exposure, which makes this combination of two recombinant salivary proteins a valid tool for screening human sera from Eastern Africa for exposure to *P. orientalis* bites.

Population structure of two sand fly genomes: *Phlebotomus papatasi* and *Lutzomyia longipalpis*

Mary Ann McDowell*¹, Frédéric Labbé¹ and the Sand Fly Genome Consortium, Eck Institute for Global Health, Department of Biological Sciences, University of Notre Dame

¹Department of Biological Sciences, Eck Institute for Global Health, University of Notre Dame

*Corresponding author: mcdowell.11@nd.edu

Keywords: *Phlebotomus papatasi*, *Lutzomyia longipalpis*, population genetics, genome

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. The cornerstone of vector-borne disease control is integrated management of vector populations. Inherent in planning these control strategies is the necessary understanding of the population structure of geographically separated vector populations as gene flow between populations and speciation events can have important epidemiological consequences, influencing vectorial capacity, insecticide resistance, and adaptation to different ecological conditions. Here we sequenced the genomes of two different phlebotomine sand flies, *Phlebotomus papatasi* and *Lutzomyia longipalpis*, that exhibit distinct distributions, behavior, and pathogen specificity. In addition, we assessed the population structure of 3 populations of *P. papatasi* collected over a large geographical range in the Middle East and North Africa and 5 populations of *L. longipalpis* from Brazil that are thought to be incipient species.

Sergentomyia schwetzi: salivary gland transcriptome, proteome and salivary enzymes

Nikola Polanska¹, Aygul Ishemgulova², Vera Volfova¹, Pavel Flegontov^{2,3}, Jan Votypka*¹, Vyacheslav Yurchenko^{2,3}, Petr Volf¹

¹Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic

²Life Science Research Centre, Faculty of Science, University of Ostrava, Ostrava, Czech Republic

³Biology Centre, Institute of Parasitology, Czech Academy of Sciences, Ceske Budejovice, Czech Republic

*Corresponding author: jan.votypka@natur.cuni.cz

Key words: Salivary gland, transcriptome, promote, enzymes, *Sergentomyia schwetzi*

During the blood feeding process, sand fly females inject to their vertebrate hosts saliva containing immunomodulatory and anti-haemostatic molecules. The saliva composition is species-specific, likely due to adaptation to the specific haemostatic pathways of their preferred host. Studies dealing with the sand fly saliva have been done so far only on *Phlebotomus* and *Lutzomyia* species. Despite of the fact that the genus *Sergentomyia* is highly abundant in many areas in the Old World, its role in human disease transmission is still uncertain. Most *Sergentomyia* spp. attack preferentially various species of reptiles, but feeding on warm-blooded vertebrates, including humans and domestic animals, has been repeatedly described, especially for *Sergentomyia schwetzi*. In the current study we analyzed *S. schwetzi* salivary gland transcriptome, proteome and salivary enzyme activities.

Illumina sequencing and *de novo* assembly of the reads and their annotation revealed 17,293 sequences homologous to other arthropods' proteins. In the sialome, we have identified all members of protein families typical for sand fly saliva: antigen 5-related, lufaxin, yellow-related proteins, PpSP15-like proteins, D7-related proteins, silk proteins, and PpSP32-like proteins. Less frequent salivary sequences included 71kDa-like protein, ParSP80-like, SP16-like, and ParSP17-like proteins. Furthermore, sand fly salivary enzymes include apyrase, hyaluronidase, endonuclease, salivary amylase, lipase A2, adenosine deaminase, pyrophosphatase, and 5' nucleotidase. Proteome mass spectrometry analysis of salivary glands identified 631 proteins, 81 of which are likely secreted into the saliva. Analysis of salivary enzymes revealed that the activity of *S. schwetzi* apyrase is significantly lower than the apyrase activity of any *Phlebotomus* or *Lutzomyia* species studied so far and that the MW of hyaluronidase is about 42 kDa.

A remarkable part of our study was focused on the mutual comparison of two *S. schwetzi* lineages derived from the same colony. These lineages were adapted for more than 30 generations for blood feeding either on mice (M) or geckos (G), two vertebrate hosts with a fundamentally different haemostatic mechanisms. Altogether 175 and 64 annotated transcripts were up-regulated in the lineage M and G, respectively. Proteomic comparison revealed 10 salivary proteins more abundant in the lineage M, whereas 69 salivary proteins were enriched in the lineage G. No difference between lineages was found for apyrase activity; on the contrary, hyaluronidase activity was significantly higher in the lineage M.

Bacterial diversity of wild-caught *Lutzomyia longipalpis* (a vector of Visceral Leishmaniasis) under distinct physiological conditions, by metagenomics and cultivable analysis.

Thais Bonifacio Campolina¹, Ana Clara Araujo Machado Pires¹ and Nagila Francinete Costa Secundino*¹

¹Instituto René Rachou/Fundação Oswaldo Cruz.

*Corresponding autor: secundinon@gmail.com

Keywords: *Lutzomyia longipalpis*; Native microbiota; Metagenomic; parasite interaction.

Leishmaniasis is a group of diseases caused by protozoa of the genus *Leishmania*. In Brazil, the sand fly *Lutzomyia longipalpis* is an important vector of visceral cutaneous leishmaniasis. Female sand flies can acquire or deliver the *Leishmania* parasite while feeding on a blood meal, which is required for their nutrition, egg development, and survival. During development within the vector, the parasite can interact with a variety of microorganisms such as fungi and bacteria. The presence of bacteria in the midgut of sand flies can influence the development and survival of the parasite. We performed a metagenomic analysis for microbiota composition and abundance in wild-caught *L. longipalpis* from Lapinha cave in Brazil. The adult insects were collected using CDC light traps and morphologically identified. *L. longipalpis* females were divided into four groups: (i) Sucrose (no blood fed), (ii) Uninfected Blood Fed; (iii) Infected Blood Fed and (iv) Gravid. Then, they were processed for metagenomics with Illumina HiSeq Sequencing to obtain the taxonomic profiles of the microbiota. The bacteria-targeted metagenomic analysis revealed different community compositions between distinct physiological stages. The amplicon oriented metagenomic profiling revealed 46 families. With the taxa indices from each experimental condition, a core composed of 6 genera was identified (*Enterobacter*, *Serratia*, *Stenotrophomonas*, *Enhydrobacter*, *Pseudomonas* and *Chryseobacterium*). Also, we described the cultivable native microbiota of *L. longipalpis* to establish if the co-culture can interfere in parasite growth. The microbiota was isolated from midguts using a culture-dependent technique and bacteria taxonomy by 16s rRNA gene sequencing. A total of 60 CFU (colony forming units) were isolated and sequenced, revealing ten bacterial genera (*Bacillus*, *Enterobacter*, *Enterococcus*, *Erwinia*, *Escherichia*, *Providencia*, *Pseudomonas*, *Staphylococcus*, *Serratia* and *Lysinibacillus*). The in vitro (lytic activity) of each one of the ten native bacteria from the *L. longipalpis* was analyzed by co-cultivation with promastigotes of *L. infantum chagasi*, *L. major*, *L. amazonensis* and *L. braziliensis*. After 24 h of co-cultivation we observed growth reduction in all parasite species. When the parasites were co-cultivated with the bacterial genus *Lysinibacillus*, all parasites of *L. (i) chagasi* and *L. amazonensis* died within 24 hours. To analyze the lytic effect of the bacterial genera in vivo, the insects were treated with Pen-strep for three consecutive days and later fed with blood infected with leishmania and the bacterial isolates individually. In the co-infection of *L.(i)chagasi*, *L. major* and *L. amazonensis* with the genera *Serratia* and *Lysinibacillus*, it was possible to observe parasite mortality with a significant difference between all tested groups in 2 and 6 days after infection. Our results detail the constituents of *L. longipalpis* native microbiota and contribute to the understanding of the bacterial community in a critical sand fly vector. This allows for further studies to better understand how the microbiota interacts with vector and parasites. Finally, the effect of *Lysinibacillus* and *Serratia* in all tested groups and assays pose them as potential candidates for blocking the infection or controlling the disease.

Funding: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fiocruz.

MALDI-TOF mass spectrometry: useful tool for species identification and blood meal analysis of Phlebotomine sand flies

Vit Dvorak*¹, Barbora Kykalova¹, Kristyna Hlavackova¹, Alexandra Chaskopoulou², Vladimir Iovic³, Bulent Alten⁴, Petr Volf¹ & Petr Halada⁵

¹ Department of Parasitology, Faculty of Science, Charles University, Czech Republic

² USDA – ARS, European Biological Control Laboratory, Thessaloniki, Greece

³ FAMNIT, University of Primorska, Slovenia

⁴ Department of Biology, Hacettepe University, Turkey

⁵ Institute of Microbiology of the Czech Academy of Sciences, Czech Republic

Corresponding author: icejumper@seznam.cz

Keywords: sand fly taxonomy, species identification, blood meal identification, protein profiling, peptide mapping

Species identification of sand flies in endemic areas is crucial to determine the significance of species in local epidemiological context. A morphological analysis of species-specific characters remains a golden standard, however, it demands laborious sample preparation and expertise in characters' assessment and often fails to give conclusive results in field samples compromised in crucial decisive morphological features. Therefore, molecular approaches complement the classical method. DNA-based analyses offer an excellent choice, however, may become laborious and expensive when vast bulks of field-collected specimens from large-scale or long-term field studies need to be processed. MALDI-TOF MS protein profiling is a protein-based technique that becomes a method of choice for arthropod identification for being rapid, low-cost and time-effective as it requires cheap and simple sample preparation plus quick analysis. Standardized protocol of specimen capture, storage and sample preparation enables to acquire species-specific protein profiles and utilize a single sand fly specimen for several purposes. It was massively deployed on sand flies collected in different countries during the Vectornet project, creating a reference database that covers 22 sand fly species. Substantial differences in protein spectra helped to reveal presence of putative cryptic *Paraphlebotomus* species in Greece. Many leishmaniasis are zoonoses with involvement of various reservoir hosts so it is crucial to determine sources of blood meals of engorged females in endemic areas to understand the significance of local vertebrates for transmission cycles of leishmaniasis. A novel method for blood meal identification was successfully developed on sand flies employing peptide mass mapping (PMM) analysis of host-specific haemoglobin peptides by MALDI-TOF mass spectrometry. It was first tested on lab-reared sand flies with correct host identification of 100% experimentally fed females until 36h post blood meal (PBM) and for 80% samples even 48h PBM, providing longer reliable blood source determination than other currently used methods where performance is quickly affected by progression of blood digestion and degradation. In a validation study on field-collected sand flies from Greece, the method unambiguously determined origin for 96% of blood meals. Moreover, it also successfully determined blood meals in specimens collected in Bosnia & Hercegovina and Croatia and stored for several years prior to the analysis. The approach allows correct identification of mixed blood meals as was demonstrated on both experimentally fed and field-collected sand flies. Simple, rapid and low-cost sample preparation procedure enables parallel isolation of DNA and subsequent deployment of DNA-based techniques. It was proven also on *Culex* mosquitoes and may be applied universally to other bloodsucking arthropods as well. Funding: This work was supported by VectorNet (Contract OC/EFSA/AHAW/2013/02-FWC1), the Grant Agency of the Czech Republic (15-04329S) and the Institute of Microbiology (RVO61388971).

Posters Presentations

Poster Session 1

Central Asian rodents as model animals for leishmaniasis: susceptibility to *Leishmania major* and infectiousness to *Phlebotomus duboscqi*

Barbora Vojtková^{*1}, Iveta Hanušová¹, Tatiana Spitzová¹, Tereza Leštinová¹, Michaela Hajková², Petr Volf¹ and Jovana Sádlová¹

¹Department of Parasitology

²Department of Cell Biology, Faculty of Science, Charles University, Prague, Czech Republic

*Corresponding author: vojtkob@natur.cuni.cz

Keywords: leishmaniasis, host model, Chinese hamsters, steppe lemmings, Djungarian hamsters

The clinical manifestation of leishmaniasis and transmissibility to sand flies depends primarily on the species of hosts and vectors, on the virulence of the parasite and also on the genetic background and the immune responses of the mammalian host. Therefore, the choice of optimal host model is very important for leishmaniasis research. Previous studies have suggested that Chinese hamsters *Cricetulus griseus* and steppe lemmings *Lagurus lagurus* are susceptible to *Leishmania* parasites, tolerate heavy infections and their histopathology is similar to human patients with visceral leishmaniasis.

The main aim was to study susceptibility to *Leishmania major* infection in three rodent species, Chinese hamsters *C. griseus*, steppe lemmings *L. lagurus* and Djungarian hamsters *Phodopus sungorus* and to compare their infectiousness to sand flies. BALB/c mice (a standard laboratory model) were used for comparison. Infections were initiated by *L. major* promastigotes isolated from the thoracic midgut of experimentally infected sand flies, or by promastigotes from the stationary phase cultures. We studied manifestation of the infection and distribution of parasites and correlated these parameters with infectiousness to *Phlebotomus duboscqi* (xenodiagnosis).

The parasite load in ears and average infectiousness to sand flies were similar for BALB/c mice and Chinese hamsters (13.5% and 11.6% of infected sand flies, respectively) although two hamsters infected more than 30% of sand flies while in mice the maximal value was 22%. Visceralisation of parasites was lower and skin lesions were less ulcerative and smaller in Chinese hamsters than in BALB/c mice. In Djungarian hamsters, the parasite load in ears, growth of skin lesions and distribution of parasites in the body were comparable with BALB/c mice, however, the lesions were more ulcerative and infectiousness to sand flies was significantly higher than in BALB/c mice (29% of infected sand flies). Steppe lemmings were equally susceptible to visceralisation as BALB/c mice, but they infected only 8% engorged sand flies as healing of skin lesion on ears was accompanied by necrosis and sand fly females had difficulty finding a suitable feeding place.

The results proved that all the Asian rodents involved in the study are susceptible to *Leishmania major*, but clinical manifestation and infectiousness to sand flies differ between species. Djungarian hamster and steppe lemmings are highly susceptible showing non-healing and healing phenotype, respectively. Chinese hamsters tolerate heavy infections better than BALB/c mice and present high numbers of parasites to feeding sand flies. Thus, Chinese hamsters appear to be the promising wild rodent models for leishmaniasis research eliciting natural response to *Leishmania* parasites.

Acknowledgements: This work was supported by ERD Funds, project CePaViP (CZ.02.1.01/0.0/0.0/16_019/0000759), the Czech Science Foundation (17-01911S) and Charles University (Research Centre UNCE 204072 and GAUK 288217).

Ultrastructural studies on Alcube virus, a new sandfly-borne virus isolated in Portugal

Fátima Amaro^{1,2}, Pedro Henriques³, Maria João Alves¹, António Pedro Alves de Matos³

¹Centre for Vectors and Infectious Diseases Research, NIH Dr. Ricardo Jorge, Portugal

²Instituto de Saúde Ambiental, Faculdade de Medicina da Universidade de Lisboa

³Centro de Investigação Interdisciplinar Egas Moniz (CiiEM), Egas Moniz, Cooperativa de Ensino Superior CRL, Portugal.

*Corresponding author: fatima.amaro@insa.min-saude.pt

Keywords: Phleboviruses, Portugal, Alcube virus

Sandfly-borne phleboviruses (genus *Phlebovirus*, family Phenuiviridae) which circulate in the Old world are included in one of three species complexes, namely Sandfly fever Naples Virus (SFNV), Salehabad Virus (SALV), and Sandfly fever Sicilian Virus (SFSV). Each species complex comprises several viruses. SFNV and SFSV can cause the usually called sandfly fever but Toscana virus (TOSV, SFNV species complex) can lead to neuroinvasive infections. Until a few years ago, viruses from the SALV group were not recognized as human pathogens but the latest seroprevalence studies showed that some of them can infect humans. Furthermore, RNA of Adria Virus (from the latter complex) was detected in an infant diagnosed with febrile seizures in Greece. Alcube Virus, (SALV species complex), was isolated from a sandfly pool collected in Arrabida region, in 2007, in the south of Portugal and until now, little is known about this new virus. The objective of this work was to study the morphology and the cytopathic effect of Alcube Virus in cell cultures for comparison of virus-cell interactions with other members of the *Phlebovirus* genus.

Subconfluent Vero E6 cells were inoculated with a viral suspension of Alcube virus. Infected Vero cells were collected 3, 4 and 5 days after infection and processed for electron microscopic examination. In brief, cells were fixed sequentially in glutaraldehyde 3% in cacodylate buffer, osmium tetroxide and uranyl acetate. After dehydration in ethanol and passage in propylene oxide, the cell pellets were embedded in an Epon-Arildite mixture, thin sectioned and studied with a JEM 1200EX.

At 3 dpi, extracellular particles attached to the plasma membrane and in the process of engulfment were observed. A few intracellular particles were seen inside vacuoles of the Golgi apparatus and in larger cytoplasmic heterogeneous vacuoles. At 4 and 5 dpi the amount of intra- and extracellular virus increased, and alteration of the endoplasmic reticulum and mitochondria were visible. The replication pattern of the virus was compatible with the described alterations of TOSV and with the previously described Arrabida virus. However intracellular changes were different, which can reflect either a different impact of the virus in cellular physiology or differences in the adaptation of the virus to in vitro replication in cultured cells.

This work was funded by an FCT project: Phleboviruses in Portugal: vectors, pathogenesis and co-infections (PTDC/DTP-SAP/0859/2014).

Adaptive gene-loss coupled to *Leishmania infantum* expansion in Brazil

Mariana C. Boité^{*1}, Philipp Schwabl², Giovanni Bussotti³, Arne Jacobs², Erich Loza Telleria⁴, Tereza Lestíková⁴, Slavica Vaselek⁴, Yara Traub-Csekö⁵, Otacilio C. Moreira⁶, Anita Freitas-Mesquita⁷, José Roberto Meyer-Fernandes⁷, Anderson Guimarães-Costa⁸, Elvira Saraiva⁸, Fernanda Morgado¹, Renato Porrozzi¹, Bjorn Andersson², Petr Volf⁴, Martin Llewellyn², Gerald F. Späth⁹, Elisa Cupolillo¹.

¹Lab. of Research on Leishmaniasis; ²IBAHCM, University of Glasgow, Glasgow, UK; ³Institut Pasteur-Bioinformatics and Biostatistics Hub-C3BI, USR 3756 IP CNRS, Paris, France; ⁴Department of Parasitology. Faculty of Science, Charles University, Vinicna 7, 128 44, Prague, Czech Republic; ⁵Lab. of Mol. Biology of Parasites and Vectors; ⁶Lab. of Mol. Biology and Endemic diseases. Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil; ⁷IBqM; ⁸Institute of Microbiology. Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; ⁹Unité de Parasitologie moléculaire et Signalisation, Department of Parasites and Insect Vectors, Institut Pasteur/INSERM, Paris, France

*Corresponding author: maricboite@gmail.com

Keywords: *Lutzomyia longipalpis*; *Lutzomyia migonei*; *Leishmania infantum*; gene-loss.

Genome analysis of 201 *L. infantum* field isolates from Americas (mainly Brazil; N= 177), Europe (N=17) and North Africa (N=2) detected a 10Kb genomic deletion of four open reading frames on chromosome 31 in a high frequency among Brazilian strains (126/177; 71%). Besides the deleted (DEL) and non-deleted (NonDEL) genotypes, heterozygous samples (HTZ) were confirmed among Brazilian strains (13/177) by cloning and qPCR. Given the stable tetrasomic state of chromosome 31, the homozygous deletion of the genomic region from all chromosomal copies is a strong indication of purifying selection, likely driven by environmental factors unique to the South American ecology. Two ORFs encode for related 3'ecto-nucleotidases (3' NT/NU), described as unique to trypanosomatid protozoa, participating in the purine salvage essential to parasite survival. 3' NT/NU activity in *Leishmania* also promotes macrophage infection and escape from neutrophil extracellular traps (NETs), a primary mechanism of host defense. Correlations to virulence are often observed in literature. We hypothesize that DEL genotypes are better adapted as a consequence of parasite-host interaction, which ultimately shaped the dispersion process. To investigate the fitness gain of the distinct genotypes in models of parasite vertebrate/invertebrate host interaction, we selected two pairs of DEL and NonDEL strains from unrelated areas where the genotypes co-circulate. Enzymatic assay confirmed a reduced 3' NT/NU activity in DEL strains compared to NonDEL. Such enzymatic deficiency could affect virulence of DEL strains. Indeed, DEL strains infected fewer macrophages in vitro and showed increased susceptibility to neutrophil NETs. These results indicate virulence attenuation of DEL strains in vertebrate hosts, though further data from natural and controlled in vivo infections are necessary to confirm this result. To achieve the wide distribution detected, DEL strains should also be able to properly infect and be transmitted by the sandflies described as vectors for *L. infantum* in Brazil. We therefore tested strain's ability to colonize *Lutzomyia longipalpis* and *Lutzomyia migonei*. Parasite loads, localization in the sand fly gut, and parasite morphology at 8 days post-infection was estimated by microscopy, using *Phlebotomus perniciosus* infected as control of Old-World sand fly species. Parasite infectivity pattern varies between the two localities tested. DEL strain from the MT region presented higher parasite count and showed a slight increase in the percentage of metacyclic promastigote forms in *Lu. longipalpis*. Strains from the PI region did not present significant differences in any parasite-sand fly combination, but infected both species at similar levels. Our results indicate that both DEL and NonDEL strains can colonize *L. longipalpis* and *L. migonei* species. Phylogenetic analysis based on the whole genome data showed a correlation of the isolates with geographic origin, independently of the presence of the deletion. Co-circulation of the genotypes occurs both in older endemic areas, such as MT, PI and in states where VL has been more recently detected, such as Santa Catarina (SC) and São Paulo (SP). The presence of the deletion irrespective of geography indicates that this mutation arises independently in the different regions and its wide distribution is thus a sign of convergent evolutionary adaptation, which points to a strong purifying selection effect. The series of phenotypic effects we have demonstrated in DEL strains may confer a net fitness gain across the parasite's life cycle. The reduced parasitemia and virulence in infections by DEL genotypes may be associated with asymptomatic or milder infections, making diagnosis difficult. Considering canine visceral leishmaniasis this would mean failure in strategies for disease control based on the detection of infected canid hosts.

Development of *Leishmania* of the subgenus *Mundinia* in sand flies and guinea-pigs

Tomas Becvar*¹, Jovana Sadlova¹, Barbora Vojtkova¹, Paul Bates², Padet Siriyasatien³ and Petr Volf¹

¹Department of Parasitology, Faculty of Science, Charles University, Czech Republic

²School of Health and Medicine, Lancaster University, Lancaster, United Kingdom

³Department of Parasitology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

*Corresponding author: tomasbecvar94@gmail.com

Keywords: *Mundinia*, *Phlebotomus*, guinea-pig, experimental infection, xenodiagnosis

Mundinia is the subgenus established in 2016 which includes five species – *L. enriettii* and *L. macropodum* are parasites of wild mammals while *L. martiniquensis*, *L. orientalis* and *L. sp.* isolated in Ghana are infective to humans. Geographical distribution of the subgenus is very large and covers all continents, except of Antarctica. *Mundinia* parasites are transmitted by two families of Diptera: Psychodidae (Phlebotominae) and Ceratopogonidae which is one of the unique features of the subgenus. *Mundinia* species were isolated from many species of mammals like guinea-pigs, kangaroos, horses, cattle and rodents, but role of these species as reservoir hosts remains uncertain.

This study combines experimental infections of sand flies and guinea-pigs using all known *Mundinia* species. Sand fly species sharing the geographical area with respective *Mundinia* species were chosen: *Phlebotomus argentipes*, *P. duboscqi* and *Lutzomyia migonei* females were fed through a chick-skin membrane on heat-inactivated rabbit blood containing 10⁶ promastigotes/ml of two strains of *L. martiniquensis* (Cu1R1 from Thailand and Lem2494 from Martinique), *L. orientalis*, *L. sp.* from Ghana and *L. enriettii*, respectively. Engorged females were dissected at various time intervals post bloodmeal (PBM) and the localization and intensity of *Leishmania* infections in guts were evaluated *in situ* under the light microscope. Furthermore, experimental infections of guinea-pigs with these *Mundinia* species were performed. Animals were injected with 10⁷ culture-derived promastigotes into both ear pinnae. Course of infections was monitored weakly, xenodiagnoses were performed and distribution of parasites in different tissues were determined *post mortem* by conventional PCR.

While *L. enriettii* and *L. sp.* from Ghana in *L. migonei* and *P. duboscqi*, respectively, were both lost during defecation, *L. martiniquensis* (Lem2494) developed moderate and heavy infections in 27 % *P. argentipes* by day 8 PBM, with colonization of the stomodeal valve in some females. Guinea-pigs infected with *L. enriettii* developed lesions on the site of inoculation, appearing at week 3 p.i. and spontaneously healing at week 11 p.i.; they were infective for sand flies by week 4 p.i. On the other hand, guinea-pigs infected with *L. macropodum*, *L. martiniquensis* (Lem2494) and *L. sp.* from Ghana did not show any signs of infection and they were not infectious to sand flies. There were no parasites detected by PCR in any of infected animals. Experiments with *L. martiniquensis* (Cu1R1) and *L. orientalis* (PCM2) are in progress.

Genetic Structure Analyses of *Lutzomyia longipalpis* and *Lutzomyia cruzi* using Next-Generation Sequencing

Aline Etelvina Casaril*¹; Diego Peres Alonso²; Karina Garcia Franco¹; Marcus Vinicius Niz Alvarez²; Suellem Petilim Gomes Barrios¹; Wagner de Souza Fernandes¹; Jucelei de Oliveira Moura Infran¹; Natália Oliveira Alves¹; Moacir Diony Gonçalves Lino Borges¹; Ana Caroline Moura Rodrigues³; Paulo Eduardo Martins Ribolla²; Alessandra Gutierrez de Oliveira¹

¹ Laboratório de Parasitologia Humana, Instituto de Biociências, UFMS;

² Laboratório de Pesquisas e Análises Genéticas, Departamento de Parasitologia, UNESP;

³ Cisne Faculdades de Quixadá.

*Corresponding author: aline.casaril@ufms.br

Keywords: high-throughput sequencing platforms, visceral leishmaniasis, *Lutzomyia longipalpis*.

Lutzomyia longipalpis is the main vector of *Leishmania infantum* in the Neotropical region, while in some municipalities of the Center-West region of Brazil, *Lutzomyia cruzi* is also involved in the transmission of this protozoan. The taxonomic status of *Lutzomyia longipalpis* complex has been widely discussed. The first named species belonging to the complex was *Lu. pseudolongipalpis* and recent studies suggest that *Lu. cruzi* could be another member of this complex. Knowledge about the genetic structure of vector insects is an essential aspect in the ecoepidemiology of metaxenic diseases, such as leishmaniasis. Thus, the aim of this study was to analyze the interpopulational genetic relations of *Lu. longipalpis* from different Brazilian regions and *Lu. cruzi*. To analyze the polymorphisms of three molecular markers and to access the population genetic levels of these sand flies, next-generation sequencing was used through the Illumina Miseq platform. To address genomic variation among *Lu. longipalpis* populations and the *Lu. cruzi* species, we used Bayesian Analysis (BA), Principal Component Analysis (PCA) and Discriminant Analysis of Principal Components (DAPC). No significant genetic differences were observed among *Lu. longipalpis* and *Lu. cruzi*. The different genetic approaches demonstrated evidence of past introgressive hybridization between *Lu. cruzi* and *Lu. longipalpis*. In addition, our results support the hypothesis that *Lu. cruzi* and *Lu. longipalpis* are sibling species. The next-generation sequencing allowed us to study different molecular markers after a single sequencing run and to evaluate population and inter-species differences on a macrogeographic scale.

Molecular taxonomic identification and genetic relationships among *Lutzomyia longipalpis* complex and closely related species inferred from cytochrome c oxidase subunit 1

Angélica Pech-May^{*1}, Sofía Lorián Moya^{*1}, María Gabriela Quintana^{1,2}, Mariana Manteca Acosta³, Oscar Daniel Salomón¹

¹Instituto Nacional de Medicina Tropical, Ministerio de Salud de la Nación, ANLIS “Dr. Carlos G. Malbrán” CONICET, Puerto Iguazú, Misiones, Argentina

²Instituto Superior de Entomología, Universidad Nacional de Tucumán- CONICET, San Miguel de Tucumán, Argentina

³Centro Nacional de Diagnóstico e Investigación en Endemo-epidemias (CeNDIE), ANLIS “Dr. Carlos G Malbrán, Buenos Aires, Argentina.

*Corresponding author: apechmay@gmail.com and sofialorian@gmail.com

Keywords: *Lutzomyia longipalpis*, *Lutzomyia cruzi*, *Lutzomyia alencari*, cytochrome c oxidase subunit 1

Identification of the species of phlebotomine sand flies present in each focus of leishmaniasis is necessary to identify the main vectors and implement vector control strategies. Similarly, the unequivocal identification of closely related species of sand flies involving vectors is highly relevant from an epidemiological viewpoint, since different species may vary in vector competence. Currently, 530 species of phlebotomine sand flies are distributed throughout North, Central and South America. In Argentina to date 38 species have been registered throughout 14 provinces. The traditional taxonomy is complex and time-consuming especially for closely related species of sand flies, furthermore, because of very subtle morphological differences between them, separation may not be possible without the use of molecular analysis. The present study examined genetic relationships and also assessed the usefulness of a 483 bp fragment of the cytochrome c oxidase I (COI) for molecular taxonomic identification among *Lu. longipalpis* complex and closely related species, *Lu. cruzi* and *Lu. alencari*. For the analysis, we used Genbank sequences of the mitochondrial COI gene of *Lu. longipalpis*, *Lu. cruzi* and *Lu. alencari*, in addition to our sequences of *Lu. longipalpis* from eight localities in Argentina (including sequences of the three haplogroups of *Lu. longipalpis*, already identified circulating in the country using ND4 and cyt *b* genes). Nucleotide sequences were aligned using clustal W incorporated in MEGA v.7. We used DnaSP v.5 to obtain the number of haplotypes by specie and haplotypes shared among close species using this fragment of the COI gene. Phylogenetic analysis was carried out with the Bayesian inference analysis implement with Mr. Bayes v.3.2. The Kimura 2-parameter genetic distance was estimated using the MEGA v.7. We identified 76 haplotypes, including three haplotypes shared among species: H5 y H12 shared between *Lu. longipalpis* (Brazil, Argentina) with *Lu. cruzi* (Brazil) and H59 shared between *Lu. longipalpis* (Brazil) with *Lu. alencari* (Brazil). The genetic distance K2p among species of phlebotomine sand flies was low, range between 0.025 ± 0.004 to 0.034 ± 0.005 . The results suggest the inefficiency of the mitochondrial COI gene in discriminating among these closely related species, the low values of K2p genetic distance possibly can be due to introgression events in sympatric zones. These findings suggest the need to search and evaluate new molecular markers when it comes to discriminate among closely related species or species complex that probable undergone recent speciation processes.

A robust phylogeny of the subgenus *Paraphlebotomus* inferred by large-scale sequencing (RAD-seq)

Véronique Lehrter¹, Astrid Cruaud², Gwenaëlle Genson², Jean-Yves Rasplus², Jérôme Depaquit*¹

¹ EA 7510 ESCAPE - USC ANSES VECPAR, SFR Cap Santé, UFR de Pharmacie, Université de Reims Champagne-Ardenne, 51 rue Cognacq-Jay, 51096 Reims, France

² INRA, UMR 1062 CBGP, Centre de Biologie pour la Gestion des Populations, Montferrier-sur-Lez, France

*Corresponding author: jerome.depaquit@univ-reims.fr

Keywords: Molecular systematics, morphology, taxonomy, Phlebotomus, Paraphlebotomus, paleobiogeography.

The subgenus *Paraphlebotomus* currently includes 14 species and includes *Ph. sergenti*, the most important vector of *Leishmania tropica*. The distribution range of the species were varying from very wide (i.e. *Ph. sergenti* or *Ph. alexandri*) to very restricted (i. e. *Ph. gemetchi* or *Ph. nuri*). Several phylogenies have been proposed based on one or two molecular markers because DNA yield from one sandfly is low which limits the number of genes that may be amplified and sequenced using Sanger approach. With NGS technologies, thousands of markers can now be routinely sequenced from single specimens and progress in the accuracy of whole genome amplification (WGA) methods can allow to circumvent input DNA amount issues. Here we successfully tested the feasibility of RAD sequencing markers from single sandflies to infer a robust phylogeny. This phylogeny shows the paraphyly of the subgenus *Paraphlebotomus* meaning we have to exclude *Ph. alexandri* from it. The North-African species *Ph. chabaudi* and *Ph. riouxi* are valid ones and early diverge. Surprisingly, *Ph. similis* is the sister species of *Ph. jacusieli* and not that of *Ph. sergenti* which is the sister species of *Ph. kazeruni*. These molecular data are compared with morphological ones and a paleobiogeographical analysis is proposed for the subgenus.

Endemism Areas of Phlebotominae in the Upper Paraguay River Basin, Mato Grosso do Sul, Brazil

S. P. G. Barrios¹, L. E. Pereira², A. E. Casaril¹, J. O. M. Infran³, W. S. Fernandes¹, E. T. Oshiro³, E. A. B. Galati⁴, G. Graciolli⁵, A. C. P. Filho⁶, A. G. Oliveira*^{1,3}.

¹ Programa de Pós-Graduação em Doenças Infecciosas e Parasitárias, Universidade Federal de Mato Grosso do Sul

² Programa de Pós-Graduação em Tecnologias Ambientais, Universidade Federal de Mato Grosso do Sul

³ Laboratório de Parasitologia Humana, Universidade Federal de Mato Grosso do Sul

⁴ Faculdade de Saúde Pública, Departamento de Epidemiologia, Universidade de São Paulo

⁵ Programa de Pós-Graduação em Ecologia e Conservação, Universidade Federal de Mato Grosso do Sul

⁶ Laboratório de Geotecnologias Ambientais, Universidade Federal de Mato Grosso do Sul

*Corresponding author: alessandra.oliveira@ufms.br

Keywords: Parsimony Analysis of Endemicity, Neotropical Region, Sandflies, Pantanal

The search for endemism areas and identification of biota is necessary to prioritize extensions for biodiversity conservation and to understand the history and relationship between biota and these regions. Biogeographic analyzes of sand flies helps the elaboration of hypotheses about the fauna composition in the regions. In addition, it can help the surveillance and conservation of risk areas for transmission of cutaneous and visceral leishmaniasis. The search for endemism areas was performed at Upper Paraguay River Basin (BAP – acronym in portuguese), located in the Mato Grosso do Sul section, Brazil. This is a site of relevant biodiversity, due to the presence of the Pantanal plateau and plain topographies. Parsimony Analysis of Endemicity (PAE) with quadrats of 3x3 latitude-longitude degrees was used to delimit the endemism areas. Maps of presence and absence distribution were produced. Cladograms of area were made through heuristic searches in the program WINCLADA/NONA. Clades sustained by the presence of more than one synapomorphy were considered as areas of endemism. A total of 64 sand fly species were reported in BAP, and 37 quadrats of 3x3 latitude-longitude degrees were obtained. The analyses resulted in a more parsimonious tree (L=212; CI=29; RI=32), which demonstrated a wide area of endemism supported by the species *Psathyromyia (Pa.) bigeniculata* and *Sciopemyia sordellii*. Five smaller areas of endemism were described: the quadrat C2, sustained by the species *Micropygomyia (Sauromyia) ferreirana* and *Mi. (Sau.) trinidadensis*; and the quadrats D4 (*Pa. campbelli* and *Viannamyia furcata*), C5 (*Ny. antunesi*, *Pa. dreisbachi* and *Pa. runoides*), E3 (*Ny. intermedia* and *Pa. scaffii*), and E4 (*Br. nitzulescui*, *Ev. orcyi*, *Mi. oswaldoi*, *Mi. vonatzingeni* and *Pi. Mamedei*). The cladograms showed possible areas of endemism at the Pantanal plateau and at the upper region of the Pantanal plain. The delimitation of these areas is important for the constant surveillance of these insects' fauna, since the protection of these areas not only maintains the region's biodiversity, but also prevents the contact of Phlebotominae vector species with human population.

Financial support: Fundect and Capes.

Impact of productive subsistence activities on the abundance dynamic of *Nyssomyia whitmani*, a *Leishmania braziliensis* vector in Misiones, Argentina

Manteca Acosta M.^{*1,2,5}, Utgés M.E.^{1,5}, Cueto G.^{3,4}, Salomón O. D.^{2,4,5}, Santini M. S.^{1,4,5}

¹ Centro Nacional de Diagnóstico e Investigación en Endemo-epidemias (CeNDIE), ANLIS

² Instituto Nacional de Medicina Tropical (INMeT), ANLIS, Ministerio de Salud de la Nación, Buenos Aires, Argentina Puerto Iguazú, Misiones, Argentina

³ IEGEBA, Instituto de Ecología, Genética y evaluación de Buenos Aires, Ciudad Universitaria, UBA

⁴ Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)

⁵ REDILA, Red de Investigación de Leishmaniasis en la Argentina

*Corresponding author: mariana.manteca@gmail.com

Keywords: *Nyssomyia whitmani*, Argentina, dynamic, Tegumentary Leishmaniasis, pigsty, henhouse

The raising of farm animals such as pigs and chickens is a productive subsistence activity developed by farm tenants of the Misiones province (Argentina), an endemic zone for Tegumentary Leishmaniasis (TL). We studied how these practices could impact and be associated to variations in *Nyssomyia whitmani*'s abundance, a *Leishmania braziliensis* vector, in a periurban zone near primary/secondary vegetation patches in the city of Puerto Iguazú. To address this objective, we selected a farm with the typical characteristics of the zone, comprising several habitats: House (H), Chicken Shed (CHS) and Deforestation Front (DF) (adjacent to Parque Provincial Península). Additionally, three Experimental Henhouses (EH) were set up near the DF to evaluate the impact of chicken breeding. Pigsty impact was not experimental; it was observed due to the owners' decision to set up one. We monthly installed REDILA light traps in H, CHS, DF, P y EH before and after its settlement, for two nights during a 4-year period (2012-2016). Data was analysed by means of generalized additive models (GAM) with a quasiPoisson error distribution. Total capture summed up 169,089 of which 68.7% was determined to species (9 genera, 15 species). Most abundant species was *Nyssomyia whitmani* (89.79%), followed by *Mg. migonei* and *Brumptomyia* spp. We observed that P installation increased *Ny. whitmani* abundance in this habitat but decreased its abundance in other habitats of the peridomicile by 51% without affecting the total mean abundance of the farm. Also, we registered that EH setting-up increased 7.5 times the mean abundance of *Ny. whitmani* in the DF. In conclusion, although these results come from an only farm, they would be explaining that the impact of a pigsty installation in the peridomicile generates a *Ny. whitmani* local population redistribution from the other habitats to the pigsty, possibly due to its greater attractivity. Additionally, the experimental trial showed that the setting-up of henhouses near the DF strongly impacts on vector abundance in the study area, not only on the redistribution inside the peridomestic area (like the pigsty) but in the total number of vectors that reach the peridomestic area. In this way, vector abundance variations caused by anthropic modifications like these productive subsistence activities, constitute epidemiological traits of TL that allow to define areas with greater risk probability of vector-human contact, generating knowledge that contributes to the developing of control strategies.

Blood feeding of *Sciopemyia microps* (Mangabeira, 1942) and *Sciopemyia sordellii* (Shannon & Del Ponte, 1927) in caves of Minas Gerais state, Brazil

Andrade, A.J.^{*1}; Marchi, G.H.¹; Santos, C.S.²; Andrade, M.C.M.³; Silva, M.A.N.²; Melo, M.N.⁴; Costa, J.C.R.³

¹ Basic Pathology Department, Federal University of Paraná, Curitiba, PR, Brazil.

² Zoology Department, Federal University of Paraná, Curitiba, PR, Brazil.

³ National Cave Research and Conservation Centre – ICMBio, Nova Lima, MG, Brazil.

⁴ Parasitology Department, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil.

*Corresponding author: andreyandrade@ufpr.br

Keywords: Food source, cavernicolous sand flies, amphibians, PCR, biodiversity.

The record of phlebotomine sand fly (Diptera: Phlebotominae) species in caves could be associated with the presence of vertebrates, source of blood feeding of some species; yet caves can be used as a natural breeding site. Several studies with cavernicolous sand flies have been conducted in Brazil in the last years were new species and genus were described, accessing bio-ecologic relationships in this ecotype, such as identifying *Leishmania* species by molecular methods. On the other hand, studies that identify the vertebrate as food source are very few. The analyzes of stomach contents of female sand flies has a great ecological and epidemiological importance given that potential reservoirs of *Leishmania* species can be identified. In addition, the blood trace detected can help identify these vertebrates in caves, which has a great call for conservation of biodiversity in these environments of intense economic exploitation. Therefore, the aim of this study is to identify the trophic preferences of species of the genus *Sciopemyia* collected in caves located in the Ferriferous Quadrilateral (FQ) at the Minas Gerais State. Specimens were collected manually near or on amphibians inside of four caves in the “Parque Nacional da Serra do Gandarela” (PNSG) and two others in the “Parque Estadual da Serra do Rola Moça” (PESRM). Males and females not engorged were clarified and mounted in Canada Balsam medium on slides for species identification. Engorged females went through the same process, but part of the abdomen was placed in vials with alcohol 70% and stored at -10°C for posterior DNA extraction, PCR amplification using specific primers and sequencing were performed and sequences were analyzed in GenBank and Barcoding of Life. All collections were authorized by the Biodiversity Information and Authorization System (SISBIO) and by the State Forestry Institute (IEF). During four collections (2016-2018) a total of 62 specimens were recorded, being 37 females and 35 males. Of the females, 23 belonged to the species *Sciopemyia microps* (Mangabeira, 1942) and 14 to *S. sordellii*. Twenty engorged females of *S. microps* were analyzed. The majority fed on *Bokermannohyla martinsi* and only one fed on *Scinax fuscovarius*. All species are included in the order Anura, family Hilydae. *S. sordellii* (n=4) fed on *B. martinsi*. This last amphibian species is endemic of the region of the FQ and is ranked as Near Threatened (NT) using criteria of the International Union for Conservation of Nature Red List. The genus *Sciopemyia* is commonly found in captures carried out inside natural caves, but this was the first study to prove females of this genus feeding on cold-blooded animals.

A historical overview on sand fly distribution and epidemiology of human and animal leishmaniosis in Eastern Europe

Mihalca A.D.*¹, Cazan C.D.¹, Sulesco T.², Dumitrache M.O.^{1,2}

¹ Department of Parasitology and Parasitic Diseases, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Cluj-Napoca, Romania

² Laboratory of Systematics and Molecular Phylogeny, Institute of Zoology, Chisinau, Republic of Moldova

*Corresponding author: amihalca@usamvcluj.ro

Keywords: *Phlebotomus* spp., *Leishmania infantum*, Sand flies, Eastern Europe, Leishmaniosis

Many canine infectious or parasitic diseases have been newly reported in the last few years as autochthonous in Eastern Europe. This is particularly evident for vector-borne diseases. The mechanisms behind the particular epidemiological pattern of parasitic and infectious diseases of pets in Eastern Europe are complex. With few exceptions, most countries from Eastern Europe are regarded as non-endemic for leishmaniosis, with sporadic cases in both humans and dogs. However, some of the sand fly vectors (*Phlebotomus neglectus*, *P. perfiliewi*) are distributed also in countries from Eastern Europe, creating the opportunity for parasite transmission. Canine leishmaniosis has been recently reported for the first time in autochthonous foci in Bulgaria, Hungary, and Romania. The aim of the current study is to synoptically review and critically analyze the distribution of the sand fly vectors and the epidemiological situation of human and animal leishmaniosis in Eastern Europe, namely in Romania, Hungary, Czech Republic, Slovakia, Bulgaria, Poland, Moldova, Ukraine (incl. Crimean Peninsula) and Belarus. Sand flies have been recorded in Romania (8 species), Hungary (4 species), Slovakia (1 species), Bulgaria (5 species), Moldova (3 species) and Ukraine (11 species). In other countries, no sand fly reports are known: Czech Republic, Belarus, and Poland. Among the countries discussed in the current paper, autochthonous cases of canine leishmaniosis have confirmed in Bulgaria, Romania, and Hungary, where they generally overlap with the known distribution of the vectors. Additionally, a multigenerational non-vectorial transmission focus was reported in Czech Republic.

mtDNA genetic variability of *Lutzomyia longipalpis* from Uruguay highlights its invasion route to the country

Cabrera Andrés* ^{1,2}, Sebastián Pita^{2,3}, Pablo Fresia^{2,4}, Viera Ana¹, González Telma¹, Lorenzo Verger⁵, Selva Romero¹, Carlos Robello², Basmadjian Yester¹.

¹ Departamento de Parasitología y Micología, Instituto de Higiene, Facultad de Medicina UdelaR

² Institut Pasteur Montevideo

³ Sección Genética Evolutiva, Facultad de Ciencias UdelaR

⁴ Unidad de Biotecnología, INIA las Brujas, Uruguay

⁵ Área de Salud Pública, Facultad de Veterinaria, UdelaR

*Corresponding author: andresmcc1@gmail.com

Keywords: *Lutzomyia longipalpis*, genetic variability, vectors and Uruguay.

Visceral leishmaniasis (VL) is a zoonotic disease caused by flagellated protozoa of the genus *Leishmania*. The disease is transmitted by dipterans of the subfamily Phlebotominae, being *Lutzomyia longipalpis* the main vector. VL affects humans and canids, being the latter a parasite reservoir. This zoonosis is endemic in the Northeast of Brazil, but VL cases have been recently reported in the Southern Cone of South America. In 2010, the Laboratory of Parasitology of the Hygiene Institute, UdelaR registered for the first time the presence of *L. longipalpis* in Uruguay. The detection of the vector, added to the regional environmental setting and the constant occurrence of new canine and human leishmaniasis cases in Mercosur countries have augmented the risk of VL in Uruguay. In 2015, the first VL cases were registered in dogs from Salto, Uruguay. Currently, the disease is considered in Salto and it has been disseminated to other cities such as Bella Unión. The genetic characterization of *L. longipalpis* populations from Uruguay can be a valuable tool to understand its invasion route and in the design of preventive measures against the progress of the disease. Here, we present the analysis of the mitochondrial ND4 gene sequences of *L. longipalpis* collected in Salto and Bella Unión, with CDC and REDILA traps, during 2013-2019. All the individuals were taxonomically classified at the species level and conserved at -20 °C for further molecular analysis. Genomic DNA was extracted using commercial kits, and then mitochondrial fragments were amplified and sequenced by Sanger approach. We found nine ND4 haplotypes that cluster together and with samples from Argentina. The comparison of ND4 sequences from different countries of Central and South America revealed six geographically concordant clusters. This result contributes to the understanding of the population structure of *L. longipalpis* at the continental scale and helps in the interpretation of the invasion route to Uruguay.

Diversity of the phlebotomine fauna (Diptera: Psychodidae) in an area of occurrence of American cutaneous leishmaniasis, State of Acre, Brazilian Western Amazonia

Andreia Fernandes Brilhante*¹; Márcia Moreira de Ávila²; Jailson Ferreira de Souza³; Priscila Bassan Sábio⁴; Marcia Bicudo de Paula⁴; Rodrigo Espíndola Godoy⁴; Vânia Lúcia Brandão Nunes⁵; Cristiane de Oliveira Cardoso⁶; Eunice Aparecida Bianchi Galati⁴

¹ Centro de Ciências da Saúde e do Desporto, Universidade Federal do Acre, Rio Branco, Acre, Brasil.

² Instituto Federal do Acre, Rio Branco, Brasil.

³ Gerência de Endemias, Prefeitura Municipal de Xapuri, Xapuri, Brasil.

⁴ Departamento de Epidemiologia, Faculdade de Saúde Pública da Universidade de São Paulo, São Paulo, Brasil.

⁵ Universidade Anhanguera-Uniderp, Campus Agrárias, Campo Grande, Mato Grosso do Sul, Brasil

⁶ Departamento de Medicina, Universidade Federal de Góias, Universidade Federal de Catalão, Catalão, Góias, Brasil.

*Corresponding author: brilhanteaf@usp.br

Keywords: Phlebotomine sandflies, rubber groves, vectors, Amazonia.

The phlebotomine fauna of the Amazon biome is considered to be one of the most diverse in the Americas, as well as in the world, presenting a large number of vectors and different species of parasites of the genus *Leishmania*. The objective of this study was better to know the phlebotomine fauna in a region of Brazilian Amazonia, endemic to American cutaneous leishmaniasis, verifying its composition and diversity. Collections were made with automatic CDC type light traps from August 2013 to July 2015, in forest, peri and intra - domiciliary areas, as well as by manual aspiration in tree trunks from January 2016 to May 2016. A total of 21,197 (14,210 females and 7,107 male) specimens were collected in CDC traps, with a richness of 60 species, distributed in four subtribes and 14 genera, the majority belonging to the genera *Nyssomyia*, *Psychodopygus* and *Trichophoromyia*. In the collections with manual aspirators 531 (186 females and 345 male) specimens were collected, the genus *Pintomyia*, *Psathryomyia* and *Viannamyia* being dominant. A large variety of sandfly species and proven and permissive vectors of *Leishmania* were observed, found in both forest and peri and intradomiciliary environments. The new records, recent descriptions and natural infections of these insects reflect the need for further studies of Acre's phlebotomine fauna and the behavior of its species, since, depending on the locality and collection method used, the abundance and diversity of species have been shown to be distinct.

Human seroprevalence of Toscana virus and Sandfly fever Sicilian virus in Setúbal district, Portugal

Carla Maia^{*1}, Lenea Campino², Nazli Ayhan³, José Manuel Cristóvão¹, André Pereira¹, Remi Charrel^{3,4}

¹Global Health and Tropical Medicine (GHMT), Instituto de Higiene e Medicina Tropical (IHMT), Universidade Nova de Lisboa (UNL), Lisboa, Portugal

²IHMT, UNL, Lisboa, Portugal

³Unité des Virus Emergents (UVE: Aix Marseille Univ, IRD 190, INSERM 1207, IHU Méditerranée Infection), Marseille, France

⁴Emerging Pathogens Institute, University of Florida, Gainesville, Florida

*Corresponding author: carlamaia@ihmt.unl.pt

Keywords: Humans, Portugal, Sandfly fever Sicilian virus, Toscana virus

The *Phlebovirus* genus (Phenuiviridae family) includes most viruses transmitted by phlebotomine sand flies. In the Old world, sand fly-borne phleboviruses belong to three antigenic complexes: The Sandfly Fever Naples Virus (SFNV) species, the Salehabad Virus (SALV) species, and Sandfly Fever Sicilian Virus (SFSV) and Corfou viruses. Although many human infections with sand fly-borne phleboviruses are either asymptomatic or influenza-like syndromes, neurovirulent Toscana Virus (TOSV) is emergent and cause outbreaks of aseptic meningitis, encephalitis or meningo-encephalitis. Up to now, TOSV have been reported in a number of Mediterranean countries. SFSV also frequently cause epidemics of febrile illness during the summer. In Portugal, human cases of TOSV meningitis along with the detection of antibodies have been reported. The presence of SFSV was suggested by the detection of antibodies in human sera in the seventies, but there is no recent data regarding the exposure of humans to this virus. The aim of this study was to evaluate the presence of antibodies against TOSV and SFSV in humans from Setúbal district, Portugal, where seroprevalence rates were reported up to 6.8% and 50.8% for TOSV and SFSV, respectively in dogs. Out of 400 human sera tested, 21 (5.3%) were seropositive for TOSV and 17 (4.3%) to SFSV. Age-dependent prevalence increase have been shown for TOSV. Humans younger than 69 years old had significantly lower positivity against TOSV. No association was observed between the presence of antibodies to both viruses. The presence of antibodies to both TOSV and SFSV in humans, together with the previous detection of both pathogens in dogs and cats, indicate that sand fly-borne phleboviruses, or closely related viruses, are circulating in Setúbal district. Our results highlight the need to increase public health awareness regarding sand fly-borne phleboviruses and to improve diagnostic tools to the rapid pathogen detection and identification.

Funding: CM and AP have the support of the Portuguese Ministry of Education and Science (via Fundação para a Ciência e a Tecnologia, I.P.), through an Investigator Starting Grant IF/01302/2015 and a PhD grant (SFRH/BD/116516/2016), respectively.

Phlebotomine sand fly survey in Algarve region, south of Portugal, 2018: molecular screening of *Leishmania* and *Phlebovirus*, and blood meal identification

Carla Maia^{1*}, Maria Odete Afonso¹, Carolina Bruno de Sousa², Nazli Ayhan³, José Manuel Cristóvão¹, André Pereira¹, Ricardo Parreira¹, César Capinha⁵, Remi Charrel^{3,4}, Lenea Campino⁶

¹Global Health and Tropical Medicine (GHMT), Instituto de Higiene e Medicina Tropical (IHMT), Universidade Nova de Lisboa (UNL), Lisboa, Portugal

²Centro de Ciências do Mar, Universidade do Algarve, Campus de Gambelas, Faro, Portugal

³Unité des Virus Emergents (UVE: Aix Marseille Univ, IRD 190, INSERM 1207, IHU Méditerranée Infection), Marseille, France

⁴Emerging Pathogens Institute, University of Florida, Gainesville, Florida

⁵Centro de Estudos Geográficos, Instituto de Geografia e ordenamento Territorial, Universidade de Lisboa, Lisboa, Portugal

⁶IHMT, UNL, Lisboa, Portugal

Keywords: Algarve, Blood meal source, CDC light traps, *Leishmania tarentolae*, Phlebotomine sand flies, Portugal

Phlebotomine sand fly-borne agents such as *Leishmania* and phleboviruses are worldwide emerging threats to public and animal health. The Algarve region, southern Portugal, has a high immigration rate from the north of Africa and the Middle East, and is the most popular tourist destination in the country. In this region a high prevalence of *Leishmania* infection in dogs and cats has been documented, together with the detection of *Leishmania infantum*, *L. major* and *Leishmania* sp. DNA in *Phlebotomus perniciosus* and *Sergentomyia minuta*. In addition, human meningitis caused by Toscana virus along with the detection of antibodies to this phlebovirus in humans and domestic animals have been recorded.

The aim of this entomological survey was to update the density of phlebotomine sand flies in Algarve region and to screen the presence of *Leishmania* and *Phlebovirus* together with identifying the blood meal sources of engorged females. Phlebotomine sand flies were collected three to six nights per month using CDC light-traps from April to November 2018. Sand flies were identified morphologically and tested for *Phlebovirus* by conventional and nested PCR assays. Females were also screened for *Leishmania* sp. by qPCR and conventional PCR. The source of blood meal of the engorged females was determined using the cytochrome b (*cytB*) partial sequence.

A total of 1628 sand flies (792 males and 836 females) were captured in 26 biotopes and identified morphologically. Specimens from *P. perniciosus*, *P. ariasi*, *P. papatasi*, *P. sergenti* and *S. minuta* were collected. The predominant species were *P. perniciosus* (71.01%) followed by *P. ariasi* (14.13%) and *S. minuta* (9.40%). 120 females were blood fed. *Leishmania tarentolae* DNA was detected in three *S. minuta*. Analysis of *cytB* DNA amplified from the blood-meals detected in the engorged females, showed that *P. perniciosus* fed on a wide range of hosts, including humans.

Despite no sand fly-borne agent pathogenic for humans and animals was detected, surveillance on *Leishmania* and *Phlebovirus* in vectors and vertebrate hosts is important as the increased migration and travelling flow elevate the risk of introduction and spread of sand fly-borne pathogens.

Funding: CM and AP have the support of the Portuguese Ministry of Education and Science (via Fundação para a Ciência e a Tecnologia, I.P.), through an Investigator Starting Grant IF/01302/2015 and a PhD grant (SFRH/BD/116516/2016), respectively.

Presence of *Lutzomyia longipalpis* in Brazilian areas without cases of visceral leishmaniasis

Paulo Silva de Almeida¹; José Oliveira da Silva¹; Marcos Antonio Batista Teixeira²; Manoel Sebastião da Costa Lima Junior³; Herintha Coeto Neitzke-Abreu*⁴

¹Regional Nucleus of Health of Dourados, Brazil

²Regional Nucleus of Health of Três Lagoas, Brazil

³Instituto Aggeu Magalhães Fiocruz (Fiocruz), Brazil; ⁴Faculdade de Ciências da Saúde, Universidade Federal da Grande Dourados (UFGD), Brazil

*Corresponding author: HerinthaAbreu@ufgd.edu.br

Keywords: Sandflies, vector control, *Leishmania infantum*, rate of infestation

Brazil is among the countries with high endemicity for leishmaniasis. The inefficiency in vector control reflects in the high number of cases. The State of Mato Grosso do Sul has as main control measure of leishmaniasis the euthanasia of diseased dogs. The objective was to investigate phlebotomines - entomological survey - in municipalities of the State of Mato Grosso do Sul (Brazil), without human cases of visceral leishmaniasis (VL). CDC type light traps were installed in environments suggestive of vector proliferation (chicken, kennel, stables and pens), during two consecutive nights in two municipalities: Bandeirantes (24 to 26 September 2018) and Sonora (25 to 28/06/2018), municipalities located in the road axis of other municipalities with moderate and intense transmission of VL. Thirty-five specimens of *Lutzomyia longipalpis* were captured in 10 points of the city of Bandeirantes, 32 males and 3 females, predominantly in the central region of the city (27 specimens). In 12 points of the city of Sonora 25 specimens of *Lu. longipalpis* were collected: 15 males and 10 females. The rate of infestation was higher in Bandeirantes (50%) than in Sonora (33%). The presence of sand flies in municipalities without human cases of VL demonstrates the need to carry out integrated control actions with environmental education, household sanitation, construction of animal shelters far from the residences, canine census survey, health education through social mobilization and survey entropy at the most appropriate periods for collecting the vector, in the months with adequate temperatures (above 25 °C).

Ecological study and risk factors for the transmission of American Cutaneous Leishmaniasis(ACL) in conservation areas - Paraná – Brazil

Cruz, Mariza F R; Galati, Eunice, A B; Cruz, Carolina F R; Marquez, Ellen S; Calderón, Celmira; Santos, Ana Paula, M E; Alcantara, Leia S; Martinhao, Maria Clara.

*Corresponding author: mfordellone@uenp.edu.br

Keywords: Phlebotominae, ACL, vectors, epidemiology, risk factors.

In the Conservation Units of the State of Paraná there are rebirth areas of preserved and degraded forests, as well as forests and ciliary reforestation. Areas that can serve as breeding sites and shelters for sandflies that frequent anthropic environments around these parks. The park surveyed in 2017 was the Mata dos Godoy State Park-Londrina-Pr (23° 27 'S and 51° 15' W longitude). It has 675.70 ha of subtropical forest, about 200 species of trees, areas of environmental preservation, is a rich genetic reserve of the South of Brazil and is home to rare tree species such as peroba, angico, cedar. In terms of its fauna, it has about 180 species of wild birds, and mammals such as hick, peccary, capybara and others. In this study, CDC type automatic traps were used to collect sandflies. The collection was done during the year 2017, five collection points were selected, sandflies were screened, clarified and identified in FSP/USP. A total of 1186 sandflies were collected of which 4 species are transmitters of the disease, especially *Nyssomyia whitmani* that corresponded to 91.48% (84,23% females), *Pintomyia fischeri* 5,73% (82,35% Fem) *Migonemyia migonei* 1,09%, *Pintomyia pessoai* 0,04 %, the others were *Pintomyia misionensis*, *Pintomyia monticola*, *Brumptomyia brumpti*, *Brumptomyia nitzulescui*, with an index below 1 %. However, the presence of the vectors does not imply the active transmission of the disease, the presence of an infected reservoir is necessary. An interview was conducted with 160 people to evaluate the risk of LTA transmission in these areas. Residents of the neighborhoods, park employees, and people who were in the area in search of leisure or research participated. 65.6% of the people never heard about the disease and did not have protective screens in their houses, 3.75% had a lesion and a scar compatible with ACL, 21.87% knew someone who had the disease, 58.12% lived at rural areas, 63.75% were at the park for recreation, 23.12% were workers, 17.5% were doing tourism or research, 85% were doing activities until 18 hours p.m. or more, and did not use insect repellents, 47.5% did activities in trails or forests and 38.75% carried out activities near lakes, rivers and waterfalls, 76.25% attended areas with organic matter and wild animals, 61.87% had pets as dogs, possible hosts, and others. It can be observed that most people are unaware of the disease and its forms of transmission and prevention, such as the presence of ACL vectors, organic matter, domestic and wild animals, absence of physical barriers such as screens in homes, and they do not use repellents or insecticides, factors that may increase the risk of transmission of the disease.

Ecological niche of *Lutzomyia longipalpis* (diptera: Psychodidae), vector of visceral leishmaniasis, in the state of Espírito Santo, Brazil

Karina Bertazo Del Carro¹, Claudiney Biral dos Santos¹, Israel de Souza Pinto¹, Blima Fux*¹ and Aloísio Falqueto¹.

¹Federal University of Espírito Santo

*Corresponding author: blimafux@yahoo.com.br

Keywords: Geoprocessing, *Lutzomyia longipalpis*, Ecological Niche Modeling.

Lutzomyia longipalpis is the main vector of visceral leishmaniasis (VL) in Brazil. The first cases of leishmaniasis were detected in two municipalities of Espírito Santo state, during the decade of 1960. Nowadays, ten municipalities are endemic, considering the current area of VL occurrence in Espírito Santo state. It is believed that the endemic migrate came from Minas Gerais state through Rio Doce valley, where the vector finds an appropriate environment for its the development. Spatial analysis has been widely used to elucidate the mechanisms of disease dispersal, in order to predict its progress to new areas and propose control measures. Geographic information systems can help to analyze the relationship between environment and health related events. Our objective was to construct an ecological niche model for *L. longipalpis* in Espírito Santo, predicting areas with possible insect occurrence, in order to alert the future presence of VL, as well as the direct resources to the priority areas. The climatic variables were listed from the WorldClim database, while the geographic variables from INPE and Geobases. The data on the presence of the transmitting species are from more than 30 years of entomological research in the state. We used the Arcgis program (Version 10.3.1) for as a tool for insertion of the selected variables and the MaxEnt algorithm for the ecological niche modeling (MNE). The area under ROC curve (AUC) was greater than 0.9, indicating that the model was excellent. Among the studied variables, the rainfall season precipitation was the one that most contributed to the generation of the model, followed by the isothermal, precipitation of the driest season, thermal seasonality, altitude, rocky outcrop and average annual temperature. The *L. longipalpis* vector was observed in 19 municipalities of Espírito Santo. The MNE predict an expansion of the propitious area for the occurrence of the insect in the Rio Doce valley, and a limited probability of extending outside this river basin.

Survey of sandfly fauna in areas of transmission of American Cutaneous Leishmaniasis (ACL), evaluating abundance, diversity and equitability indices under influence of hydroelectric plants in the Paranapanema River, Paraná

Cruz, Mariza F R*; Galati, Eunice, A B; Cruz, Carolina F R; Marquez, Ellen S; Calderón, Celmira; Santos, Ana Paula, M E; Alcantara, Leia S; Martinhao, Maria Clara.

*Corresponding author: mfordellone@uenp.edu.br

Keywords: Phlebotominae, sandfly, American tegumentary leishmaniasis, epidemiology.

An epidemiological study was carried out to evaluate the factors that determine the transmission of ACL. The study was carried out in the urban, peri-urban and rural areas of the city of Itambaracá, including Vila Rural and the localities of Porto Almeida and São Joaquim do Pontal, the latter two near the area impacted by the Canoas I and II hydroelectric plants on the Paranapanema River. A total of 3187 phlebotomines were captured. The predominant species were *Nyssomyia neivai* (34.36%), *Pintomyia pessoai* (32.57%), *Migonemya migonei* (11.61%), *Nyssomyia whitmani* (8.82%) and *Pintomyia fischeri* (2.73%) all with disease transmission capacity. For *Ny. neivai*, there were predominance of males and for the other vector species, females prevailed, with significant statistical difference ($p < 0.001$). The most abundant species, according to the index of abundance of the standardized species, were *Ny. neivai*, *Pi. pessoai*, *Ny. whitmani*, *Br. brumpti*, *Mg. migonei* and *Pi. fischeri*. The highest frequencies and species diversity were found in the locality of Porto Almeida (PA), with more preserved forests, followed by São Joaquim do Pontal (SJP) and Vila Rural (VR), with more degraded forests. The presence of sand flies was observed in all sampled locations, with five species with a vector capacity of ACL agents, especially *Ny. Neivai*, with a higher frequency near the peridomiciliary areas and the higher frequencies of sandflies, including the vectors, were verified in areas close to forests, and in the most preserved, *Pi. pessoai* predominated.

Baseline sand fly data and monitoring of cutaneous leishmaniasis in an endemic area close to the Toachi-Pilaton hydroelectric dam: Joint initiative between HIDROTOAPI and LEMMT-USFQ

Andrés Carrazco^{1,2}, Kimberly Caranqui¹, Felipe Ilivichuzca³, Juan Pablo Jativa³, Renato León*¹

¹Laboratorio de Entomología Médica & Medicina Tropical, LEMMT, Colegio de Ciencias Biológicas y Ambientales, Universidad San Francisco de Quito, Cumbayá, Quito, Ecuador

²Instituto Nacional de Investigación en Salud Pública- Dr. Leopoldo Izquieta Pérez (CZ9), Quito, Ecuador

³Corporación Eléctrica del Ecuador, CELEC EP, Unidad de Negocio Hidrotoapi, Quito, Ecuador.

*Corresponding author: rleon@usfq.edu.ec

Key words: sand flies, hydroelectric dams, leishmaniasis, CDC light traps

The construction of hydroelectric dams in rural areas contributes to local and national development, generates substantial income and savings and helps to produce own resources in developing countries. However, it also produces environmental change, deforestation and thus, changes in the habitats and natural cycles of many species, including sand flies (Diptera: Psychodidae), vectors of Cutaneous Leishmaniasis (CL). In Ecuador, the western foothills of the Andes mountains, including the area of the construction of the Toachi-Pilaton Hydroelectric Dam (TPHD), has been historically endemic for CL. Nevertheless, the sand fly species present and vector species involved in CL transmission in this region have been poorly investigated. Herein, we present preliminary results of an entomological baseline study of the sand fly species and monitoring of the disease in an area close to the TPHD. Entomological collections using CDC light traps were carried out in forests and peridomestic areas in 2014 revealing at least 7 sand fly species (from 48 specimens collected) present in the region including the potential CL vector species *Lu. trapidoi* (8%). Other species collected were *Lutzomyia serrana* (36%), *Lu. hartmanni* (33%), *Lu. aclydifera* (11%), *Lu. dreishbachi* (8%), *Lu. camposi* (2%), *Lu. shannoni* (2%). Further sand fly surveillance in 2017, collected the following 6 species (from 38 specimens): *Lutzomyia serrana* (63%), *Lu. hartmanni* (21%), *Lu. trapidoi* (7%), *Lu. aclydifera* (3%), *Lu. camposi* (3%) y *Lu. abonnenci* (3%). The TPHD local health center admitted 2 recent cases of CL in 2019, what called the attention of the importance of this project. The first case was a child, male, aged 10, with a single ulcer lesion located at the nose in his face. The second case, a 35 years old female, with a single ulcer lesion on the right arm. Clinical Diagnoses were carried out at TPHD and patients were sent to the Augusto Egas government Hospital (MSP 23D01) located in Santo Domingo de los Colorados city for diagnosis confirmation through microscopic examination of ulcer samples. In both patients, ulcer lesions were successfully treated at the hospital with intramuscular injections of Meglumine Antimoniate (Glucantime®). It is intended that this study continues after TPHD starts its operation, tentatively at the end of 2019. Future entomological and disease surveillance at TPHD and its surroundings will allow to compare data obtained before and after the dam's construction and beginning of its operation, to identify changes in sand fly species composition and abundance and shifts in disease transmission. Furthermore, will also help to identify the best strategies to minimize the long-term effects that this emblematic national facility may have in the transmission of CL. This initiative is the result of a joint-effort between a public company (Hidrotoapi) and a private institution (Universidad San Francisco de Quito) and could serve as a reference, for implementing similar studies in other regions of Ecuador, where hydroelectric dams and mining or oil exploitation operations, may have an impact on the natural cycle of vector-borne diseases and on vector populations, and thus on the risk of disease transmission.

Leishmaniasis in Galapagos: Past reported cases and, entomological data to address if local transmission is possible

Anaconda Salas Navarrete¹, Sandra Enríquez², Alexandra Ángulo¹, Marilyn Cruz³, Patricia Álvarez Alvarado⁴, Ana María Gómez⁵, Juan Ochoa⁴, Renato León^{*6}

¹Escuela de Medicina Veterinaria, Facultad de Salud, Universidad de las Américas, Quito-Ecuador

²Instituto de Investigación en Salud Pública y Zoonosis, Universidad Central del Ecuador. Quito, Ecuador.

³Agencia de Regulación y Control de la Bioseguridad y Cuarentena para Galápagos ABG, Avenida Baltra, Puerto Ayora, Santa Cruz, Galápagos, Ecuador.

⁴Hospital Oskar Jandl, Coordinación 5, Ministerio de Salud Pública. Av. Juan Pablo II y Jaime Roldós Aguilera, Puerto Baquerizo Moreno, San Cristóbal, Galápagos, Ecuador

⁵Hospital República del Ecuador, Av. Baltra, esquina Av. Charles Darwin, Puerto Ayora, Santa Cruz, Galápagos, Ecuador

⁶Laboratorio de Entomología Médica & Medicina Tropical, LEMMT, Colegio de Ciencias Biológicas y Ambientales, Universidad San Francisco de Quito, Cumbayá, Quito, Ecuador

*Corresponding author: leon@usfq.edu.ec

Keywords: sand flies, islands, leishmaniasis, transmission risk, CDC light traps

Cutaneous leishmaniasis (CL) has been described in 23 of the 24 provinces of Ecuador, except in Galapagos. However, local health authorities there have referred two cases of CL. Both patients were temporary Galapagos residents. The first case, reported in 2017, was a male patient, aged 21, from Santo Domingo de los Tsachilas, with 3 ulcer lesions (one in the left arm and two at the posterior thorax); he was admitted to the Oscar Jandl hospital on San Cristobal Island. The second case, a 49 years old male from Babahoyo, Los Rios province, had 2 ulcer lesions on the right leg and foot. He was admitted to the República del Ecuador Hospital on Santa Cruz Island. Diagnoses were carried out by microscopic examination of ulcer samples at the INSPI in Guayaquil and in a private health center, respectively. Ulcer lesions were successfully treated locally through intramuscular injections of Meglumine antimoniate (Glucantime®). To further investigate these clinical reports and assess the risk of local CL transmission, snapshot CDC light traps collections were carried out outside and inside of residences in Puerto Ayora, Bellavista, and Santa Rosa on Santa Cruz Island and near residences in Puerto Baquerizo Moreno (San Cristobal Island), and Puerto Villamil (Isabela Island). Over 8,000 insects were identified into the Diptera order and in at least 10 families including Ceratopogonidae, Chironomidae, Sciaridae, Culicidae, and Psychodidae (subfamily Psychodinae). No sand flies (Diptera: Psychodidae: Phlebotominae) were found in the collections. Although climatically, the Galapagos Islands could allow for the establishment of invasive sand fly species, we believe that the risk is very low due to: (i) short flight range and high sensitivity to sudden temperature changes, (ii) wind on the shores of the islands that would not allow for flying insects to get onto land, (iii) the specificity of its ecological niche, (iv) specificity of reservoirs to feed on, and (v) strict control by local authorities to monitor the possible entry of materials into which sand fly larvae / pupae could be accidentally included. The absence of CL vectors in the islands suggests that the two past disease reports most likely corresponded to imported cases from the continent. Nevertheless, local health personnel must be acquainted with the disease and its diagnosis and treatment, considering the possibility of new cases in the future due to increasing commerce and the flow of people to Galapagos from CL endemic areas, especially coastal Ecuador.

Leishmanicidal activity of Ishpink and Moringa

Franklin Espinosa¹, Patricio Rojas-Silva^{1,2}, Tatiana Mosquera³, Michelle Parra³, Héctor Flor³, Olalla Barreiro-Costa², Lourdes Orejuela⁴, Sonia Zapata*¹

¹ Instituto Microbiología USFQ

² Centro de Investigación Biomédica UTE.

³ Universidad Politécnica Salesiana.

⁴ Colegio de Ciencias e Ingenierías USFQ

*Corresponding author: szapata@usfq.edu.ec

Keywords: *Leishmania mexicana*, *Ocotea quixos*, *Moringa oleifera*, IC50

Leishmaniasis is a tropical neglected disease caused by a protozoa of the genus *Leishmania* and causes a spectrum of diseases. The range of clinical manifestations include cutaneous syndromes associated with the parasite infection and the visceral variant.

According to the World Health Organization (WHO) the disease affects the poorest people and is associated with a weak immune system, lack of financial resources, is linked to environmental changes such as deforestation and urbanization. Around of 1 million new cases and some 26000 to 65000 deaths occur annually. According to the Epidemiological Report of the Americas of the Pan American Health Organization, March 2019 only 49,1% of the cases progressed to clinical cure, 0.03% resulted in death and 50.7% the progression is unknown /unspecified probably due to the abandonment of the treatment for its adverse effects.

The study of plants is widely practiced for the control of bacterial, fungal and parasitic infections, including leishmaniasis. Several compounds have been isolated from plant extracts as antileishmanicidal agents, almost all of them have not been studied. *Ocotea quixos* Lam. (Lauraceae) and *Moringa oleifera* Lam (Moringaceae) are the best-known plants use to investigation for its medicinal, antibacterial, antifungal and antiprotozoal activities. With the high percentage of treatment failure and the increase of pentavalent resistance. The objective of this study is to evaluate three leave-extracts, *Ocotea quixos* ethanolic extract (OQEX), *Moringa oleifera* aqueous (MOAX) and *Moringa oleifera* ethanolic extract (MOEX) against *Leishmania mexicana* as a therapeutic alternative of leishmaniasis cutanea.

All tests were performed in triplicate and the fifty inhibitory concentration (IC50) were made for linear regression log(inhibitor) vs. response (three parameters) (Prism version 8.1.2). A significant effect of the MOEX extract on promastigotes of *Leishmania mexicana* was observed in the screening tests, adjusted P value (<0,0001). In the dose-response assay the IC50 for *L. mexicana* was 7.874 (CI: 0.8873 - 44.59) and the IC50 for the Raw cells was 12.95 (CI: 7.255 - 23.95). The selectivity index (SI) was 1.64. This result shown no selective toxicity on leishmania and Raw cell line

It is interesting to note that some biological activities of natural products can be evaluated against promastigotes of *Leishmania* spp. According to the literature several compounds with leishmanicidal activity have been isolated such as isothiocyanate and polyphenol extracted from Moringa's ethanolic fraction may have biological activity.

Poster Session 2

Multi-scale environmental influences on sandfly community structure in Amazonia

Chavy*^{1,2}, A. Kocher^{3,4}, S. Tirera¹, C. Leroy¹, M. Huguin¹, J-F. Guégan⁴, G. Prévot² & B. de Thoisy¹

¹Laboratoire des Interactions Virus-Hôtes, Institut Pasteur de la Guyane, Cayenne French Guiana

²Laboratoire des Ecosystèmes Amazoniens et Pathologie Tropicale, EA3593, Medicine Department, Université de Guyane, Cayenne, French Guiana

³CNRS, Université Toulouse III Paul Sabatier, IRD, UMR-5174 EDB (Laboratoire Évolution et Diversité Biologique), Toulouse, France

⁴Unité Mixte de Recherche MIVEGEC, Université de Montpellier, IRD, CNRS, 911 avenue Agropolis, 34394 Montpellier cedex 5, France

Corresponding author: achavy@pasteur-cayenne.fr

Keywords: community, environmental factor, ecology, Amazonia

Cutaneous leishmaniasis is common in South America, with a cycle of disease transmission mainly sylvatic, involving many species of sandfly vectors and mammalian hosts. In addition, human activities such as deforestation, agriculture, urban expansion are quickly evolving in the Amazon basin with ever increasing pressure on the forest. This great diversity of species makes it difficult to identify the vectors of the disease, and to predict the impact of these ecosystem changes on hosts and vectors and subsequently on the transmission cycle of the disease. In Colombia and Brazil, urbanization of the transmission cycle has been observed in some cases, showing that vectors and the parasite could be highly adaptive. Highlighting the factors that influence and structure host and sandfly communities could improve our understanding of the ecology of the cycle and can facilitated the prediction of new outbreaks of disease in humans. One way to address the issue of putative vectors ecology is a community ecology approach. Few studies focused on the structure of the sandflies communities and their variations in space along a gradient of anthropogenic disturbances. We expected to evaluate the influence of different multi-scale environmental variables along an anthropogenic gradient on the composition, the structure and the infectious status of sandfly species. We conducted this study in French Guiana, where the forest is still preserved but where forestry activities are growing. We trapped sandflies in 8 forest sites using CDC Light Traps. Of the 8 sites, two free of anthropogenic disturbances served as control sites. For the 6 other sites three points were distributed along a gradient of anthropogenic disturbances, with an area highly disturbed, moderately disturbed and a lightly disturbed. This first step allowed to have an intermediate spatial scale, through which we will be able to study the impact of anthropogenic factors on communities. Then, for each of these 3 points, we placed seven light traps along a transect of about 500 meters. This transect will allow studying the impact of the environment on micro-scale communities. Sandflies were pooled by 50 and the identification of sandflies species and leishmania occurrence were made by NGS with Hiseq Illumina using the marker for the 16S RNA ribosomal and minicircle, respectively. In total more than 27,000 sandflies were collected from a total of over 1000 nights / traps spread across 730 communities. Preliminary results indicate that the 730 communities combined comprise 15 different genera. Of these 730 communities 124 appear to be infected by Leishmania. As the factors that structure these communities are mainly linked to human activities, there is a real risk of urbanization and "peridomestication" of the cycle of the disease. Indeed, in French Guiana, the pressure on the environment has been accentuated in recent years favoring the contact of humans with infected mammalian hosts. The description of community structures involved in the transmission of cutaneous leishmaniasis in French Guiana will allow understanding and possibly predicting future epidemiological events and ecological condition favoring vectors.

Phlebotomine in transitional area, Chaco, Argentina. Meteorological variation

Rosa, J.R.*¹; Szelag, E. A¹; Quintana, M. G^{2,3,4}; Tourn, E¹ and Salomón, O. D^{2,4}

¹Instituto Medicina Regional-IMR-UNNE. Nodo REDILA-Red de Investigación de la Leishmaniasis en Argentina. Resistencia, Chaco. Argentina

²Instituto Nacional de Medicina Tropical (INMeT), Misiones, Argentina

³Instituto Superior de Entomología, Universidad Nacional de Tucumán, Tucumán. Argentina ⁴CONICET. C.A.B.A, Argentina

*Corresponding author: juan_rosa05@yahoo.com.ar

Keywords: Sandflies, Abiotic factors, Eco-regions.

Phlebotomine are distributed in Argentina to 29°NL in ecoregions with leishmaniasis records where Chaco province (24°28'S, 58°63'W) is included. It is divided in two sub-regions: eastern Humid Chaco with humid Atlantic rainfall pattern (1200 mm/year) in summer and autumn, and western Dry Chaco with semiarid continental rainfall pattern (500–800 mm/year) in autumn and winter. This pluviometric gradient marks a transitional area with gallery forests where monthly abundance and diversity of Phlebotominae according to climatic variables were studied. It was carried out from 2011 to 2014 in Tres Isletas town (26°20'S, 60°25'W) and Pampa del Indio town (26°02'S; 59°56'O) in two houses with leishmaniasis records. Five CDC light trap was installed at indoor or veranda, peridomicile and forest (base and canopy of one tree) during 12h on crepuscular times for 2 or 3 consecutive nights. Monthly Relative Humidity (RH%), accumulated monthly Precipitations (PPT), Minimum Temperature (Min.T), Maximum Temperature (Max.T) and monthly average Temperature (Med.T) were requested to the nearest weather stations. A total of 44,938 Phlebotominae were collected: *Migonemyia migonei* (72,21%), *Nyssomyia neivai* (18,89%), *Evandromyia cortelezzii/sallesi* (4,11%), *Ev. cortelezzii* (2,54%), *Ev. sallesi* (1,34%), *Brumptomyia brumpti* (0,82%), *Ev. aldafalcaoae* (0,04%), *Psathyromyia bigeniculata* (0,02%), *Ev. sp.* (0,02%) and *Ev. termitophila* (0,01%). The first three species were captured all months and the rest were not frequent. *Migonemyia migonei* (n=32,448) predominated in both sites, every year in autumn and spring on March/September (2011-12) and March/October (2012-14). *Nyssomyia neivai* (n=8,491) in autumn and summer on March (2013) and December (2013). *Cortelezzii* complex (*Ev. cortelezzii* and *Ev. sallesi*) in autumn and summer on April/December (2011) and spring and summer on October/November (2012). The other species (<1%) were captured on summer, autumn and spring. Climatic averages were: 28°C (Máx.39°C-Mín.21°C), 45% (RH) (Máx.100%-Mín.10%) and 185mm (PPT). On winter, minimal record was 9°C-10°C and Max. 22°C-25°C, 15mm (PPT). Correlations of monthly abundance of the most abundant and frequent species, with meteorological variables of the same month of captures and with one (LAG-1) and two (LAG-2) previous months recorded: *Mg. migonei* had a higher correlation with PPT ($r_s = 0.48$ $p < 0.023$) and monthly Mean T ($r_s = 0.46$ $p < 0.029$). (LAG -1): Correlation with maximum and monthly average temperature and negative correlation with the average monthly RH. (LAG-2): Correlation with monthly Max.T). *Nyssomyia neivai* correlated only with maximum monthly RH ($r_s = 0.54$ $p = 0.008$). (LAG-1): Positive correlation with temperature, which is higher with the monthly average temperature. (LAG-2): Correlation with Min. T and negative correlation with minimum monthly RH. *Cortelezzii* complex showed no correlation with any of the meteorological variables of the same month of capture. (LAG-1) and (LAG-2): Correlation only with the maximum monthly temperature. Finally, *Mg. migonei* and *Ny. neivai* were captured all seasons and *Cortelezzii* complex in autumn, spring and summer. The climatic variables highlighted, according to the species, a different correlation with temperature, rainfall and humidity which could influence the abundance and frequency but without eliminating them and maintaining the transmission hypothesis conditioned by the abiotic variables.

Seasonal distribution of *Lutzomyia longipalpis* in Santo Tomé, Corrientes, Argentina

Villarquide ML^{*1,4}; Utgés ME^{2,4}; Leporace M¹; Rilo MC¹; Santini MS.^{2,3,4}

¹Laboratorio de Control de Vectores Entomológicos de Importancia Sanitaria (LaCVEIS) Fundación Barceló sede Santo Tomé, Corrientes.

²Centro Nacional de Diagnóstico e Investigación en Endemo-epidemias (CeNDIE).

³Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

⁴Red de Investigación de las Leishmaniasis en la Argentina (REDILA).

*Corresponding author: lvillarquide@barcelo.edu.ar

Keywords: sandfly, *Leishmania infantum*, Visceral Leishmaniasis, Corrientes, Longitudinal study.

Lutzomyia longipalpis, the vector of *Leishmania infantum* in America was recorded for the first time in 2008 in Santo Tomé. The aim of this study was to evaluate the temporal distribution of *Lu. longipalpis* and its variation, caused by environment modifications that were made by the residents (elimination of hens) and the correlation between the average temperature and the average humidity of the peridomiciles.

Eleven categorized places were selected according to the abundance of *Lu. longipalpis*, one site of Null Abundance (without sandflies), 5 of Low Abundance (1-10 sandflies), 2 of Intermediate Abundance (11-30 sandflies) and 3 of High Abundance (30 or more sandflies). The captures were made using REDILA light traps that were put in sites where the dog sleeps or in henhouses from 5 PM to 8 AM for three consecutive nights, once in a month for 2 years (2016-2017). The changes in the environment made by the residents such as the elimination or incorporation of farmed animals (hens) were recorded in a follow-up chart. The temperature and the humidity were recorded every night in each sampling site with a digital thermo-hygrometer with minimum and maximum values (TFA®).

Total capture of *Lu. longipalpis* summed up 7743 individuals in 2016 and 5721 in 2017. During two years of study, 3 sites eliminated the henhouses where the captures were made, making it possible to observe changes in the abundances of *Lu. longipalpis* that were maintained in time. One of the sites of Intermediate Abundance, with a range of 115-292 sandflies for 7 months, turned into Low with a range of 0-3 *Lu. longipalpis* during the remaining 17 months. Likewise, two other High Abundance sites also converted to Low Abundance: one with a range of 231-311 individuals during 2 months to a range of 0-14 sandflies for the remaining 22 months; and the other one, with a range of 0-1313 *Lu. longipalpis* during 12 months to a range of 0-2 individuals during the remaining 12 months. The other sampling sites remained without modification during the whole study, not showing great abundance variations of *Lu. longipalpis*.

In 2016, the lowest abundance of *Lu. longipalpis* (n=1) was observed in July with a temperature of 3.5°C, showing a correlation with the minimum average temperature (r=0.88, p=0.01) and the maximum (r=0.72, p = 0.02) for that year. In 2017, the minimum abundance *Lu. longipalpis* (n = 11) was observed in June with a temperature of 12.6 °C, resulting in a correlated abundance with the average minimum temperature (r= 0,61, p = 0.05).

The henhouses provide feeding and breeding sites for *Lu. longipalpis*, and their elimination decreases the abundance of the vector. Nevertheless, the presence of *Lu. longipalpis* is permanent throughout the year. The warm climate with high minimum temperatures even in winter, contributes to the constant presence and the possibility of human-vector contact.

Nichtemeral rhythm of sand flies (diptera: psychodidae) in campo grande, Mato Grosso do Sul, Brazil

Moacir Diony Gonçalves Lino Borges^{1*}; Aline Etelvina Casaril²; Suellem Petilim Gomes Barrios²; Wagner de Souza Fernandes²; Jucelei de Oliveira Moura Infran¹; Elisa Teruya Oshiro²; Everton Falcão de Oliveira³; Alessandra Gutierrez de Oliveira^{1,2}

¹ Universidade Federal de Mato Grosso do Sul, Instituto de Biociências, Programa de Pós-Graduação em Biologia Animal, Campo Grande, MS, Brasil.

² Universidade Federal de Mato Grosso do Sul, Instituto de Biociências, Campo Grande, MS, Brasil.

³ Universidade Federal de Mato Grosso do Sul, Instituto Integrado de Saúde, Campo Grande, MS, Brasil.

*Corresponding author: dionylino@hotmail.com

Keywords: *Lutzomyia longipalpis*, nichtemeral rhythm, sand flies, Campo Grande.

Studies on the nichtemeral rhythm of sand flies are of paramount importance in public health, since they may contribute to the orientation of prophylactic measures to control the transmission *Leishmania* parasites. This study aims to investigate and describe nichtemeral rhythm of sand flies (Diptera: Psychodidae) in Campo Grande, Mato Grosso do Sul, Brazil. The sand fly collections were carried out from May to December 2018 in two forest areas: one in the urban area (Rita Vieira Neighborhood: 20° 33' 40.03" S; 54° 36' 06.44 " W; 541 m above sea level), and the other in the rural area (São João Farm: 27° 37' 10.73" S; 54° 31' 57.33" W; 543 m above sea level). Monthly collections were performed during 24 hours uninterrupted in Shannon traps in both sand fly sampling sites. A total of 147 sand flies were collected. Of these, 120 in the Rita Vieira neighborhood – urban region – and 27 were captured in the São João farm, in the rural area. The species identified in the urban area were; *Lutzomyia longipalpis*, *Pintomyia christenseni*, *Nyssomyia whitmani*, *Brumptomyia avellari*, *Evandromyia lenti* and *Psathyromyia bigeniculata*. In the rural area, the species identified were the same species above, with the exception of *Nyssomyia whitmani* and with the addition of *Psathyromyia punctigeniculata*. *Pintomyia christenseni* was the most prevalent species in the rural area (66.66% of the total specimens) and *Lutzomyia longipalpis* was the most prevalent in the urban area, with higher activity from June to August (99.04%), and deserves greater attention because it is the main vector of *Leishmania (Leishmania) infantum* in the Americas. The daily activity occurred between 5 pm to 4 am for urban area and from 5 pm to 2 am for rural area. We noted that no have differences between forest areas inside urbanized or preserved environment.

Where does *Nyssomyia whitmani* breed? Habitats and conditions that favour the vector in rural areas of Misiones, Argentina

Manteca Acosta M.^{* 1, 2, 5}, Utgés M. E.^{1, 5}, Cavia R.^{3, 4}, Santini M. S.^{1, 4, 5}, Salomón O. D.^{2, 4, 5}.

¹ Centro Nacional de Diagnóstico e Investigación en Endemo-epidemias (CeNDIE), ANLIS, Ministerio de Salud de la Nación, Buenos Aires, Argentina

² Instituto Nacional de Medicina Tropical (INMeT), ANLIS, Ministerio de Salud de la Nación, Puerto Iguazú, Misiones, Argentina

³ IEGEBA Instituto de Ecología, Genética y Evolución de Buenos Aires (UBA-CONICET), Buenos Aires, Argentina

⁴ Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET),

⁵ REDILA, Red de Investigación de Leishmaniasis en la Argentina.

*Corresponding author: mariana.manteca@gmail.com

Keywords: immature stages, breeding sites, environmental variables, emergence, Tegumentary Leishmaniasis.

The knowledge of phlebotomine biology has been increasing in the last decades worldwide, though information about immature stages in nature is still lacking. The objective of this study was to identify the habitat characteristics that associate with higher numbers of emergent adults of *Nyssomyia whitmani*, the Tegumentary Leishmaniasis (TL) causal agent vector in a rural zone of Puerto Iguazú, Misiones, Argentina. To achieve the goal, 8 farms were sampled for emergent phlebotomine by setting up 41-69 emergence traps in the two principal natural breeding sites detected in a previous study: Below House (BH), space underneath stilt houses; and wooden Chicken Shed (CHS). Active traps were inspected and relocated every 40 days, between November 2015 and April 2016 equivalent to 4 phlebotominae life cycles approximately. The following habitat variables were registered: structural characteristics of buildings, tree cover, distance between habitats, number of inhabitants and domestic animals. A total of 84 emergent adult phlebotominae (92.9% *Ny. whitmani*) were registered in 1716 emergence traps in all the period: 22 in BH and 62 in CHS. Phlebotominae emergence was detected in 7 of 8 farms (87.5%). After multiple regression analysis using GLMM we found that the highest abundances of emergent *Ny. whitmani* occurred when the number of chickens was high, being more pronounced in CHS compared with BH habitat (coef=1.32, df=55, p=0.0004). We also observed higher abundances at short distances from the forest (coef=-1.88 df=55, p=0.02), and minimum abundances in March and April (coef =-1.32, df=55, p=0.006, Tukey contrast, p<0.05). This study indicates that these habitats, besides having the major adult abundances as revealed in previous studies, also represent breeding sites for immature insects, making them epidemiologically relevant for managing/control LT actions/campaigns. Additionally, it allows the recommendation of relocation for houses and chicken sheds away from the forest front between the limits of each farm.

Aspects of the phlebotomine fauna (Diptera: Psychodidae) and of the infection by *Leishmania* spp. in an area of high incidence of Cutaneous Leishmaniasis in Rio Branco, Acre

Márcia Moreira de Ávila*¹; Andréia Fernandes Brilhante²; Eunice Aparecida Bianchi Galati²; Reginaldo Peçanha Brazil³

¹ Instituto Federal do Acre (IFAC)

² Faculdade de Saúde Pública da Universidade de São Paulo

³ Fundação Oswaldo Cruz

***Corresponding author:** marcia.avila@ifac.edu.br

Keywords: Amazon, Phlebotomine, Leishmaniasis, infection

Phlebotomines (Diptera: Psychodidae) are insects of medical importance due to their involvement in the zoonotic cycle of transmission of *Leishmania* to vertebrates. The objective of this study was to detect *Leishmania* infection in these insects, collected in rural and urban environments of the municipality of Rio Branco, State of Acre. The sandflies were collected using Shannon and HP light traps, from December 2014 to January 2016. To investigate the natural infection by *Leishmania*, DNA samples were extracted from pools of sandfly females and submitted to the polymerase chain reaction (PCR) using the primers for the ITS1 and *cyt b* regions; *Leishmania* species were identified by blast. A total of 2,517 individuals were collected, and 43 species were identified. *Trichophoromyia auraensis* (839; 33.35%), *Trichophoromyia* ssp. (537; 21.35%) and *Evandromyia saulensis* (187; 7.43%) were the most abundant species. *Leishmania* DNA was sequenced with amplification of thirteen samples and the presence of *Leishmania* (*Vianna*) *braziliensis* was confirmed in: *Trichophoromyia auraensis* (1 female) *Evandromyia saulensis* (2 females), *Evandromyia walkeri* (2), *Psychodopygus llanosmartinsi* (1), *Pintomyia nevesi* (2), *Psychodopygus davisii* (1), *Psychodopygus ayrozai* (1), *Psathyromyia aragaoi* (1) *Nyssomyia antunesi* (1), *Evandromyia infraspinosa* (1). Only in one sample of *Psychodopygus ayrozai* was the sequence similar to that of the *Leishmania* (*Vianna*) *guyanensis*. In the microscopic analysis, five species of *Evandromyia saulensis* showed flagellate forms in the posterior gut, suggestive of *Leishmania* infection, whose rate was 2.4%. These results demonstrate a high proportion of sandflies infected with *Leishmania*, common in the Amazon region.

Spatial distribution of immatures and adults of *Lutzomyia* spp. in a periurban focus of leishmaniasis in the Caribbean region of Colombia

Horacio Cadena*¹, Luis Gregorio Estrada², Andrés Vélez¹, Edgar Ortega², Luz Adriana Acosta¹, Rafael José R. Vivero¹, Sandra I. Uribe³, and Eduar E. Bejarano²

¹ Programa de Estudio y Control de Enfermedades Tropicales -PECET. Universidad de Antioquia, Medellín – Colombia.

² Grupo de Investigaciones Biomédicas. Universidad de Sucre, Sincelejo – Colombia.

³ Grupo de Investigación en Sistemática Molecular. Universidad Nacional de Colombia, Medellín – Colombia.

*Corresponding author: horaciocadena@gmail.com

Keywords: *Lutzomyia*, Phlebotomine immature, Vegetation, Leishmaniasis, Periurban

Phlebotomine sand flies are responsible for the transmission of diseases of medical importance, such as bartonellosis, some arboviruses and leishmaniasis. The distribution and abundance of vectors as well as the reporting of new cases of leishmaniasis are affected in combination by climatic factors and some human activities such as deforestation, road construction and unplanned urbanization. The replacement of natural vegetation by agricultural crops has also been proposed as modulators of the distribution and composition of the vectors and a risk factor for leishmaniasis. In Colombia, in the last two decades several studies have described the risk of infection by *Leishmania* associated with deforestation and the replacement of native vegetation by coffee, cocoa and fruit tree crops. The objective of this study was to describe the spatial distribution of adults and the immature forms of sand flies in the periurban vegetation of an endemic area for leishmaniasis. The study was carried out in three zones of the periurban area of the municipality of Ovejas, department of Sucre, located in a tropical dry forest life zone. The capture of adults at rest, the search for immatures and characterization of the vegetation was carried out at a maximum distance of 200 meters from the dwellings every two months during 2013-2014. Adults at rest were collected on trees with a trunk circumference greater than 30 cm for three consecutive days. The search for immatures was conducted on the base of trees, litter and pens of domestic animals. Plots of 100 m² each were designed for the floristic characterization of the vegetation in the three study zones. The spatial representation of the sand flies, immature forms and vegetation were constructed with the program QGIS version 3.4.4-Madeira. The Shannon-Wiener and Jaccard indexes were used to determine the diversity and similarity of the vegetation. A total of nine field trips were made and 717 phlebotominees were collected at rest in 13 arboreal species. Of the 10 species of sand flies that were collected at rest, *Lu. evansi* and *Lu. cayennensis* were the dominant species with 58 and 46% respectively. *Cordia alba*, *Guazuma ulmifolia* and *Ceiba petandra* congregated 86.7% of the sand flies collected at rest. 230 immature forms were recovered from 14 tree species. 66% (n = 152) of the immature were isolated from *Cordia alba* of which *Lu. evansi* accounted for 40% (n = 93). The plant community of the three zones was heterogeneous. 95% of the vegetation identified as breeding and resting sites of the phlebotomine was located 100 meters from the houses. The highest frequency and relative dominance of species corresponded to *Cordia alba* in zones 1 and 3. In this context, the chance of the population coming into contact with sand flies may be greater due to the proximity of the vegetation. The high density of *Cordia alba* and in turn breeding site of *Lu. evansi*, recognized vector of visceral and cutaneous leishmaniasis, could favor the intra and peridomiciliar transmission of leishmaniasis. These results suggest considering the role of *Cordia alba* and its proximity to housing as an ecological marker of risk in this peri-urban focus and include peridomiciliar vegetation in the disease control program.

Emergence of Toscana virus and other sandfly-borne phleboviruses in arid biogeographical areas of central Tunisia

Dachraoui K¹, Fares W¹, Barhoumi W¹, Derbali M¹, Cherni S¹, Fraihi W¹, Chelbi I¹, Zhioua E*¹.

¹ Pasteur Institute of Tunis, Laboratory of Vector Ecology, Tunis, Tunisia.

*Corresponding author: elyes.zhioua@gmail.com

Keywords: Phleboviruses, Sand flies, Toscana virus.

Sand flies are vectors of *Leishmania* parasite and phleboviruses affecting humans and animals. In this work, we aimed to investigate phlebovirus circulating in a focus of zoonotic visceral leishmaniasis (ZVL) located in Central Tunisia during 2014, 2015 and 2016. The research of phleboviruses was performed by nested PCR in the polymerase gene and dead sand flies were identified to species level. Sand flies of the subgenus *Larroussius* mainly *Phlebotomus perfiliewi*, *Phlebotomus perniciosus*, and *Phlebotomus longicuspis* were predominant in this ZVL focus. In 2014, of a total of 117 pools, 4 were positive, yielding a minimum infection rate (MIR) of sand flies with phleboviruses of 0.2% (4/1932). Phylogenetic analysis performed using partial nucleotide and amino acid sequence in the polymerase gene showed that these phleboviruses belonged to four different clusters corresponding to Toscana virus (TOSV), Saddaguia virus (SADV), Sandfly Fever Sicilian Virus (SFSV) and Utique virus (UTIV). Also, TOSV was isolated from two positives pools among 60 pools of sand flies collected in 2015, yielding a MIR of 0.11% (2/1740). In 2016, TOSV was also detected in one pool among 18 pools, yielding a MIR of 0.22% (1/444). Phylogenetic analysis showed that the Tunisian strain of TOSV belonged to the sublineage A. We report the first detection and isolation of TOSV from sand flies collected from Central Tunisia. TOSV can thus be the cause of meningitis or fever of unknown origin, not only in the humid, subhumid and semi-arid bioclimatic stages, but also in the arid zones of central Tunisia.

Irrigation in the arid regions of Tunisia impacts the abundance of sand flies' population and *Leishmania infantum* infection

Walid Barhoumi¹, Wasfi Fares¹, Saifedine Cherni¹, Mohamed Derbali¹, Khalil Dachraoui¹, Ifhem Chelbi¹, Marcelo Ramalho-Ortigao³, John C Beier⁴, Elyes Zhioua*¹

¹ Pasteur Institute of Tunis, Laboratory of Vector Ecology, Tunis, Tunisia

² Faculty of Sciences of Bizerte, University of Carthage, Bizerte, Tunisia

³ Department of Entomology, Kansas State University, Manhattan, KS, USA

⁴ Department of Public Health Sciences, University of Miami Miller School of Medicine, Miami, FL, USA

*Corresponding author: elyes.zhioua@gmail.com

Keywords: Zoonotic visceral leishmaniasis; irrigation; sandflies; *Leishmania infantum*

The current spread of zoonotic visceral leishmaniasis (ZVL) throughout arid areas of Central Tunisia is a major public health concern. The main objective of this study is to investigate whether the development of irrigation in arid bio-geographical areas in Central Tunisia have led to the establishment of a stable cycle involving sand flies of the subgenus *Larroussius* and *Leishmania infantum*, and subsequently to the emergence of ZVL. Sand flies were collected from the village of Saddaguia, a highly irrigated zone located within an arid bio-geographical area of Central Tunisia by using modified Centers for Diseases Control (CDC) light traps. Morphological keys were used to identify sand flies. Collected sand flies were pooled with up to 30 specimens per pool according to date and tested by nested Polymerase Chain Reaction (PCR). DNA sequencing from positive pools was used to identify *Leishmania* spp. A total of 4915 sand flies (2422 females and 2493 males) were collected from Saddaguia in September and in October 2014. Morphological identification confirmed sand flies of the subgenus *Larroussius* to be predominant. PCR analysis followed by DNA sequencing indicated that 15 pools were infected with *L. infantum* yielding an overall infection rate of 0.6%. The majority of the infected pools were of sand fly species belonging to subgenus *Larroussius*. Intense irrigation applied to the arid bio-geographical areas in Central Tunisia is at the origin of the development of an environment capable of sustaining important populations of sand flies of the subgenus *Larroussius*. This has led to the establishment of stable transmission cycles of *L. infantum* and subsequently to the emergence of ZVL in Central Tunisia.

Natural infection of *Lu. longipalpis* (Cembrene-1) with *Leishmania infantum* in a VL focus of the East region of São Paulo State, Brazil

Fredy Galvis-Ovallos^{1*}, Mariana Dantas da Silva¹, Vanessa Gusmon da Silva¹, Eunice Aparecida Bianchi Galati¹, João Augusto Franco Leonel², Trícia Maria Ferreira de Sousa Oliveira³, Maria Anice Mureb Sallum¹, Claudio Casanova⁴

¹Departamento de Epidemiologia, Faculdade de Saúde Pública-Universidade de São Paulo-USP ²Programa de Pós-Graduação em Epidemiologia Experimental Aplicada às Zoonoses, Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo

³Faculdade de Zootecnia e Engenharia de Alimentos (FZEA)

⁴Superintendência de Controle de Endemias (SUCEN)

*Corresponding author: galvisfregao@gmail.com

Keywords: *Lutzomyia longipalpis* complex, natural infection, *Leishmania infantum*, visceral leishmaniasis, Cembrene-1.

In Brazil, visceral leishmaniasis (VL) is a zoonosis widely distributed that shows different epidemiological patterns. This disease is caused by *Leishmania infantum* which is transmitted by *Lutzomyia longipalpis* infected females. In São Paulo state, differences in the ecoepidemiology of VL associated to the occurrence of two different populations of *Lu. longipalpis* complex have been observed. In the East region of SP state, the Cembrene-1 population has been identified in peri urban and rural landscapes. In Valinho, located in the eastern region of SP, VL canine cases have been recorded, which motivated the development of the investigation of the phlebotomine fauna in the city to identify the sandfly species and evaluate the circulation of *Le. infantum*. Sandfly captures were undertaken with CDC traps (18:00 to 06:00 hours) and manual aspiration (18:00 to 21:00 hours) in three domiciles. Four captures were undertaken from April/2018 to December/2018. The sandflies captured were transported to the Public Health Entomology laboratory of the FSP/USP and were dissected to identify the species and check the infection status. Females were dissected in a drop of PBS and observed in microscopy (400X). DNA of the samples was extracted and submitted to PCR using as target the Internal Transcribe Spacer 1 (ITS-1). The PCR-amplified products were sequenced, and the generated sequences were submitted to BLAST search in the GenBank database. Five species were identified *Expapillata firmatoi*, *Migonemyia migonei*, *Nyssomyia whitmani*, *Pintomyia fischeri* and *Lu. longipalpis*. Of 57 females of *Lu. longipalpis* captured, 47 were dissected and intense infection by promastigotes were observed in two samples. Flagellates were in the midgut, characterizing a suprapylarian infection. The sequences obtained matched with *Le. infantum*. A natural infection rate of 4.2% was observed. Natural infection rates could vary depending on the prevalence of the host infection and the method of detection (parasitological or molecular) considering the differences in sensibility, with the higher rates obtained using molecular techniques. Because a small sample was analyzed, our results demonstrated the circulation of VL agent of and a high infection force, suggesting a high risk of transmission in the studied area. This reinforces the necessity of entomological surveillance activities and the control measures adoption of to prevent the occurrence of human cases.

Detection of DNA of *Leishmania infantum* in *Nyssomyia intermedia* in a new focus of visceral leishmaniasis in São Paulo State, Brazil. What is the role of this sandfly in VL transmission?

Claudio Casanova^{1*}, Gabriela Motoie², Maria de Fátima Domingos, Fredy Galvis-Ovallos³, Vanessa Gusmon da Silva³, Mariana Dantas da Silva³, ¹Eunice Aparecida Bianchi Galati³.

¹Superintendência de Controle de Endemias (SUCEN)

²Instituto Adolfo Lutz (IAL)

³Faculdade de Saúde Pública-Universidade de São Paulo-USP.

*Corresponding author: casanovaclaus@gmail.com

Keywords: *Nyssomyia intermedia*, natural infection, *Leishmania infantum*, visceral leishmaniasis, vector.

In Brazil, visceral leishmaniasis (VL) is a zoonosis caused by *Leishmania infantum* and transmitted to mammals - including dogs and humans - mainly by the bite of infected females of *Lutzomyia longipalpis*. However, the absence of this sandfly in captures undertaken in a recent focus of canine and human VL in the coastal municipality of Guarujá (SP), where *Nyssomyia intermedia* was the most abundant species, raise the suspicion that this species is involved in the transmission cycle of this disease. So, in the present study we investigated the natural infection of the sandfly species of this locality. Sandflies were collected from December 2016 to October 2018 with CDC traps installed in the peridomicile of the dwelling where human cases had occurred as well as in the peridomicile of neighboring houses. The females were identified and pooled in 1.5 ml tubes containing from one to 31 specimens, of the same species and date of capture. The DNA was extracted from 39 pools by QIAamp DNA mini kit (Qiagen). PCR was carried out with primers 150/152 (*Leishmania spp*), RV1/RV2 (*L. donovani* complex) and Lu.18S 1S/Lu.18S AR (*Lutzomyia* 18S rRNA gene). A total of 1,203 specimens were collected: 1,091 (90.7%) of *Ny. intermedia*, 63 of *Migonemyia migonei* (5.2%), 37 of *Psathyromyia pascalei* (3.1%), 11 of *Pintomyia fischeri* (0.9%), and one of *Psychodopygus ayrozai*. *Nyssomyia intermedia* was the only species present in all the months of collection and showed a bimodal time distribution with one peak during the hottest and rainy season (from December to February) and another during the coldest period (May to August). Of the 723 females of *Ny. intermedia* collected, 352 were stored in 27 pools containing from 01 to 31 specimens and were analyzed by PCR. The DNA of *L. (L.) infantum* was detected in two pools of *Ny. intermedia* - one pool containing 17 specimens collected in January 2017 and another with 20 specimens collected in June 2018. The housekeeping gene of *Lutzomyia spp.* was amplified in all the DNA samples. No *Leishmania* DNA was detected in the remaining pools containing females of *Mg. migonei*, *Pi. fischeri*, *Pa. pascalei* and *Ps. ayrozai*. The occurrence of canine and human cases of VL in the absence of *Lu. longipalpis*, coupled with the predominance of *Ny. intermedia* in the peridomiciles - including where two human cases had been notified - and the presence of DNA of *L. infantum* in two pools of females, support the hypothesis of the participation of this sandfly species in the transmission cycle in this area. However, ecological and laboratory studies to evaluate the vector capacity and competence of *Ny. intermedia* are necessary. The molecular detection of *L. infantum* in *Ny. intermedia*, a species widely distributed in Brazil, may indicate that this scenario could be present in other regions where this species occurs and reinforce the importance of studies associated with permissive sand fly vectors.

Seasonal assemblage of Phlebotominae species, Tucumán, Argentina.

AD Fuenzalida^{1,2}, LM Diaz Briz², JM Direni Mancini^{2,3,4}, GA Rodriguez², OD Salomón^{1,3,4}, MG Quintana^{1,2,3,4*}

¹Instituto Nacional de Medicina Tropical (INMeT), ANLIS “Dr. Carlos G. Malbrán”, MSyDS, Misiones, Argentina

²Instituto Superior de Entomología, Universidad Nacional de Tucumán, Tucumán. Argentina.

³CONICET. Argentina.

⁴REDILA-Red de Investigación de las Leishmaniasis en Argentina.

*Corresponding author: gabrieladelaquintana@gmail.com

Keywords: *Migonemyia migonei*, compleción *cortelezzii* *Evandromyia cortelezzii*– *Ev. sallesi*, *Pintomyia salomoni*, abundance

In the Northwest Argentina, the main vector implicated in the transmission of cutaneous leishmaniasis (CL) is *Nyssomyia neivai* (Pinto). In Tucumán province, the endemic area of CL, *N. neivai* is found mostly in the south. The last major outbreak was recorded in 2003 and was associated with the gallery forest of the Marapa River, which flows from the Escaba Dam, located 14 km away from the outbreak area. The objective of this work is to study the seasonal assemblage of Phlebotominae species in the area of influence of Escaba Dam, where a colony of insectivorous bats is found. The region is belonging to the subtropical forest of the Yungas and transitional zones with xerophytic forests. The captures were made in the 2013 with REDILA-BL light traps, for three consecutive nights three times by season. A total of 12 traps were placed in forest and gallery forest environments. Bivariate analysis was carried out to determine differences between the abundances of Phlebotominae and the seasons (autumn-winter: A-W, spring-summer: Sp-Su). To compare the abundance of each species between the sampling sites and the seasons, Friedman’s test is carried out. A total of 730 individuals belonging to four species were captured: *Migonemyia migonei* (França) (68%), compleción *cortelezzii* *Evandromyia cortelezzii* (Brethes) – *Ev. sallesi* (Galvao & Coutinho) (17%), *Pintomyia salomoni* (Quintana & Fuenzalida) (9%), *Nyssomyia neivai* (0.2%), without identification due to material condition (6%). *Nyssomyia neivai* was not considered in the analysis for its low abundance. *Mg. migonei* was the dominant species throughout the year, and together with *Ev. cortelezzii* showed peaks of greater abundance in the Sp-Su seasons. On the other hand, the abundance of *Pi. salomoni* was greater at seasons A-W. Regarding to the difference between the sampling sites, C3 (forest), C4 (gallery forest) and C10 (gallery forest), were the most abundant for all species. In the sites where *Pi. salomoni* and *Ev. cortelezzii* were present, their abundances were similar, while *Mg. migonei* was the most abundant in all of them. The diversity of the community of Phlebotominae in the Escaba Dam is greater than in the area where the outbreak of 2003 occurred. In this area, *Pi. salomoni* was found and described for the first time, simultaneously captured in the province of Jujuy, 300 km away. It should be noted that this species is closely related to two Bolivian species in environments with the presence of bats, leaving many questions about the biology and epidemiological role of this species.

Distribution, Abundance and Infection of *Lutzomyia longipalpis*, vector of Visceral Leishmaniasis in three Municipalities of the Department of Caaguazú, Paraguay

Nilsa González Brítez*^{1,2}, Lidia Boy^{1,2}, María Ferreira^{1,2}, Analía Ortiz¹, Enmanuel Céspedes², Mónica Ruoti¹, Gladys Estigarribia¹, Sheila Benitez³.

¹ Asociación de Funcionarios de la Universidad Nacional de Caaguazú.

² Instituto de Investigaciones en Ciencias de la Salud, Universidad Nacional de Asunción.

³ Departamento de Zoonosis, V Región Sanitaria, M.S.P. y B.S.

*Corresponding author: gbritez.nilsa@gmail.com

Keywords: *Lutzomyia longipalpis*, Leishmaniasis visceral, *Leishmania* sp., risk zone.

Lutzomyia longipalpis, is the main vector of Visceral Leishmaniasis (VL) in Latin America, and is a complex of species with wide distribution throughout the continent with adaptations to different habitats. Several areas of Paraguay, including the Department of Caaguazú, are considered endemic for leishmaniasis. The aim of this study was to determine the distribution, abundance and infection of *Lu. longipalpis* in the urban and peri urban areas of three districts of the department of Caaguazú, Paraguay. For collecting the insects, 108 CDC traps were placed in housing of urban and peri urban areas in the San José, Caaguazú and Coronel Oviedo districts. The insects were identified with the taxonomic key of Young and Duncan and the females were subjected to DNA extraction and PCR method to determine the infection with the parasite. The presence of sandflies was detected in 9.3% of the total traps placed and 41 sandflies were captured, 97.5% corresponding to the species *Lutzomyia longipalpis* and 2.5% to *Nyssomyia whitmani*, of which 34.2 % were females. The infection with *Leishmania* sp. was determined by PCR and was positive in 7.1% of the sandflies. The distribution of traps with phlebotome-infested by municipalities was registered for San José (13.6%), Coronel Oviedo (11.4%) and Caaguazú (4.8%). It is concluded that the vector is widely distributed in the study area and the determined infection confirms the risk of frequent transmission of leishmaniasis, which represents an area of potential risk for the appearance of human visceral leishmaniasis. Finally, the authors consider that it is necessary to continue with the integrated management measures of epidemiological surveillance and to initiate a community health education strategy for the control of this vector.

Financial Support: CONACYT (Consejo Nacional de Ciencia y Tecnología), Asunción, Paraguay.

Sandflies fauna (Diptera: Psychodidae) in a border area of Paraguay and the influence of environmental variables on the vector density of *Lutzomyia longipalpis*

Nilsa González-Britez*^{1,2}, Lidia Boy¹, Enmanuel Céspedes¹, María Ferreira¹, Andrea Gimenez², Nidia Martínez³, Antonieta Rojas de Arias⁴.

¹ Instituto de Investigaciones en Ciencias de la Salud, Universidad Nacional de Asunción.

² Facultad de Ciencias de la Salud, Universidad Nacional del Este.

³ Servicio Nacional de Erradicación de Paludismo SENEPA.

⁴ Centro para el Desarrollo de la Investigación Científica, CEDIC.

*Corresponding author: gbritez.nilsa@gmail.com

Keywords: *Lutzomyia longipalpis*, phlebotomine, Leishmania spp., Paraguay

Phlebotomines (Diptera: Psychodidae: Phlebotominae) are considered important for public health because they are involved in the transmission of the protozoa *Leishmania* spp., which cause Leishmaniasis, a zoonosis considered one of the six most important tropical diseases in the world. The objective of this work was to investigate the presence of phlebotomines in the department of Alto Paraná and to study the influence of environmental variables on the vector density of *Lutzomyia longipalpis*. Entomological monitoring was carried out between 2014 and 2017 in three municipalities of Alto Parana, Paraguay. This component involves three-night sampling with light traps on grids of 400 x 400 m in a transversal survey and a longitudinal sub-sample. A total of 180 small light traps were placed in urban and peri urban areas, weather conditions were monitored. The sandflies were composed of five genera and six species totaling 332 individuals. The dominance of *L. longipalpis* (73%) was determined, followed by *Nyssomyia whitmani* (22%), related to the transmission of *L. visceral* and *L. cutaneous* respectively. *L. longipalpis* was found more abundantly in the urban and peri urban sites, without showing a correlation between its frequency and the temperature of the environment, but not with the relative humidity found in the sampling areas. The distribution of *L. longipalpis* in peri urban and urban sites of Alto Paraná clarifies a new profile of distribution and expansion of the disease, due to possible changes of vector ecotopes, previously restricted to the wild environment

Financial Support: IDRC-Canada Project #107577, PAHO

Checklist and distribution maps of the phlebotomine sandflies of Bulgaria

Ognyan Mikov¹, Ivelina Katerinova², Markéta Grešová³, Vít Dvořák³, Filiz Günay⁴, Özge Erisoz Kasap⁴, Vladimir Ivović⁵, Petr Volf³, Bülent Alten⁴

¹ Department of Parasitology and Tropical Medicine, National Centre of Infectious and Parasitic Diseases, Sofia, Bulgaria

² Parasitology Section, National Diagnostic Science-and-Research Veterinary Medical Institute, Sofia, Bulgaria

³ Department of Parasitology, Charles University, Prague, Czech Republic

⁴ Department of Biology, Faculty of Science, Hacettepe University, Ankara, Turkey

⁵ Department of Biodiversity, Faculty of Mathematics, Natural Sciences and Information Technologies, University of Primorska, Koper, Slovenia

*Corresponding author: mikov@ncipd.org

Keywords: sandfly fauna, Bulgaria

Phlebotomine sandflies are known to transmit parasites, bacteria and viruses that affect humans and animals in many countries worldwide. During the XXth century, they were considered to transmit human visceral leishmaniasis and sandfly fever in Bulgaria. Early studies on species composition in the first half of the century revealed the presence of five species of genera *Phlebotomus* and *Sergentomyia* in the country.

There has been a 60-year gap in the sandfly research with no data on them between 1950 and 2010, when the medically important insects of primary interest were the mosquitoes. Fourteen different regions of Bulgaria were sampled for sandflies during the period 2011-2014 using different light traps and CO₂ traps and 991 specimens were collected. Moreover, thirteen regions were sampled between 2015 and 2016 during the VectorNet field campaign in the country and 1268 specimens were collected. Most of them were identified to species level using morphological keys and confirmed by PCR and MALDI-TOF assays. Published data on the distribution of the Bulgarian sandfly species collected over the period 1909-2011 were reviewed. Both historical and contemporary collection localities for each species were plotted using the Universal Transverse Mercator (UTM) grid system on a contour map.

An updated checklist, together with UTM maps showing the known distribution records of the ten species of sandflies collected in Bulgaria are presented. The species list includes *Ph. alexandri*, *Ph. balcanicus*, *Ph. kandelakii*, *Ph. neglectus*, *Ph. papatasi*, *Ph. perfiliewi* s.l., *Ph. sergenti*, *Ph. tobbi*, *S. dentata* and *S. minuta*.

Field collection of sand flies as one of the funded projects of local participatory budget in Torrelodones municipality (Madrid), Spain

Gálvez R^{1,2*}, Hernández S², Montoya A², Miró G².

¹Department of Specific Didactics. School of Education and Teacher Training. Universidad Autónoma de Madrid, Spain.

²Department of Animal Health, Veterinary Faculty, Universidad Complutense de Madrid, Spain

*Corresponding author: rosa.galvez@uam.es

Keywords: Citizens, field work, sand flies, participatory budget.

Leishmaniasis caused by *Leishmania infantum* has been in the spotlight since in 2009 when the largest human leishmaniasis outbreak occurred in Europe that also affected the south-west area of the Madrid region (Arce et al., 2013, Euro Surveill 18, 20546). Moreover, Madrid region has been traditionally considered endemic for *L. infantum* infection in dogs (Gálvez et al., 2010, Vet parasitol 169, 327-334). Torrelodones, a municipality located in the northwest of the Madrid region, has made efforts to implement participatory budget investment policies. According to the 2016 census, the population of the territory was 23,123 inhabitants and presented a population density of 1053.44 inhabitants per km². Participatory budgeting offers citizens the opportunity to take part in the municipal management. Every year local government set aside up to 100,000 euros as a separate line item for participatory budget. Neighbours are encouraged to build up their own proposals and to vote among all the selected. Anyone over 16 years old who is registered in the municipality can participate. A proposal entitled: “Study about insect populations which transmits Leishmaniasis in Torrelodones municipality” was one of the eight projects that obtained a favorable report from municipal technicians. Following this step, the eight feasible proposals were voted and our project ended in the third place. With 124 votes, our project was funded under the 2019 participatory budget campaign (<https://participaentuspresupuestos.torrelodones.es/>). It is worth stressing that our project was the only one related with science. In fact, the others were mostly related with municipality infrastructure investments.

The aim of the present project is to study sand fly populations in Torrelodones municipality, with special attention paid to the confirmed vectors of *L. infantum*: *Phlebotomus perniciosus* and *P. ariasi*. The specific objectives set out are: **1)** To analyze sand fly abundance and the spatial distribution throughout the municipality. The study area will be surveyed using sticky traps (A5-size paper coated with castor oil). The sampling sites will be evenly distributed across a 17-square grid (1 km each side). At each sampling site, 20 sticky traps will be set. Based on point abundance data for the two sand fly vectors of leishmaniasis in the region (*P. perniciosus* and *P. ariasi*), GIS models based on environmental/meteorological variables will be constructed to predict sand fly spatial distribution. **2)** To address seasonal trends during the whole sand fly activity period (from the end of May to mid October), the animal protection center for dogs and cats will be surveyed. This place is selected for being a good indicator of sand fly density in previous years. The specimens will be collected using CDC miniature light traps fortnightly. The knowledge of temporal trends of sand flies will allow planning preventive measures to avoid sand flies’ bites and minimize disease transmission. It should be noted that such a municipal initiative together with science awareness of neighbours has propelled the funding of this project that will be carried out in 2019 sand fly activity period.

Harnessing student citizen science in and around the classroom to study sand fly populations in Madrid (Spain)

Gálvez R^{1,2*}, Gollerizo A¹, Clemente M¹.

¹Department of Specific Didactics. School of Education and Teacher Training. Universidad Autónoma de Madrid, Spain

²Department of Animal Health, Veterinary Faculty, Universidad Complutense de Madrid, Spain

*Corresponding author: rosa.galvez@uam.es

Keywords: Citizen science, field work, sand flies, students.

BRITEC (Bringing research into the classroom) is an ERASMUS+ project funded by European Commission from 2018 to 2021 under the Key action 2 (Cooperation for Innovation and Exchange of Good Practices). The BRITEC project proposes introducing research into classrooms through Citizen Science activities, co-designed between schools and research institutions. The aim is to increase the interest of young students in science through the direct involvement in scientific processes, interaction with scientists and the use of high-quality educational materials.

Spanish partners of Universidad Autonoma de Madrid led a subproject whose objective is to involve students in citizen science in and around the classroom to study sand fly populations in Madrid region (Spain). This subproject includes three complementary blocks of activities, which build on each other to develop a set of exemplary practices and guidelines for the implementation of Citizen Science in the classroom and to ensure their large-scale dissemination and uptake:

- To design light traps with air gate handmade by the own students in a Fablab (Fabrication Laboratory). The objective of this block is to develop a DIY (do it yourself) trap model easily replicated and made from recycled materials.

- Sand fly specimens will be captured at different time-points within the sand fly activity season (from the end of May to mid-October). We plan that pupils will choose several places around the school to collect sandflies. Geographical location (GPS data) and photos of traps will be made by the students using smartphones. Apart from collecting sand flies, students will be trained to distinguish between sandflies and other similar flying insects and to identify different species.

- Investigator in charge will analyze sandfly abundance and the spatial distribution of different species, with special attention paid to the competent disease carriers.

Student citizen science not only produces novel scientific research results that may inform public health decisions, but also students participate in the scientific process itself by observing, gathering and processing the data.

Ecological and seasonal aspects of sand fly populations in Austria

Edwin Kniha¹, Julia Walochnik¹, Adelheid G. Obwaller², Wolfgang Poepl³, Petr Volf⁴, Petr Halada⁵ & Vit Dvorak^{1*4}

¹Institute of Specific Prophylaxis and Tropical Medicine, Center for Pathophysiology, Infectology and Immunology, Medical University of Vienna, Austria.

²Federal Ministry of Defense, Division of Science, Research and Development, Austria.

³Department of Dermatology and Tropical Medicine, Military Medical Cluster East, Austrian Armed Forces, Austria.

⁴Department of Parasitology, Faculty of Science, Charles University, Czech Republic.

⁵Institute of Microbiology of the Czech Academy of Sciences, Czech Republic.

*Corresponding author: icejumper@seznam.cz

Keywords: sand fly ecology, Central Europe, Austria, blood meal, phylogeny

In Europe, sand flies are widely distributed in Mediterranean countries where transmission of *Leishmania infantum* is endemic. Their occurrence in Central Europe was long time overlooked as the Alps were assumed to form a natural barrier for their northward expansion. The first findings of *Phlebotomus mascittii*, a suspected but unconfirmed vector species of *Leishmania* spp. in Germany in 1999 and subsequently in Austria and the bordering countries Slovenia, Hungary and Slovakia are rather recent. Thus, research on Central European sand fly populations was long time neglected and knowledge is very limited. Temperature and humidity are considered to be the most important factors for sand fly occurrence and dispersal. Austria plays a key role in Central European sand fly distribution as the climatically favored Danube valley could function as an ecological corridor to connect Eastern populations in Slovakia, Hungary and Slovenia with Western populations in Germany. Only low trapping numbers of a single species *Phlebotomus mascittii* have been reported in Austria and the knowledge on the distribution and associated ecological factors is scarce. Therefore, a long-term study was conducted during the summer months 2018 to assess sand fly activity and associated ecological factors in Austria. Four different locations in Lower Austria and Styria were sampled for four consecutive nights a week, from 27 June to 14 September with standardized CDC miniature light traps. Overall, 271 sand fly specimens were trapped, of which 214 (79%) were female and 57 (21%) were male. The average female/male-ratio was 1/3.8 and significantly biased towards females ($\chi^2=45.7$, p-value<0.001). Trapped specimens were identified by morphological and molecular. Despite four other sand fly species occurring in bordering countries, all recorded specimens were *Ph. mascittii*. Several mitochondrial markers were used to understand phylogenetic differences between Austrian sand fly populations and their relations to populations in other countries. Detailed associations between sand fly activity and climatic parameters including temperature, relative humidity, wind speed, precipitation, air pressure and lunar illumination at studied sites were evaluated. Sand flies were observed to be active at a wide range of climatic conditions. All specimens were screened for the presence of *Leishmania* and endosymbiotic *Wolbachia* bacteria by PCR. Blood meals of engorged specimens were analyzed in parallel by MALDI-TOF MS peptide mapping and sequencing analysis of standard genetic markers (cytB, PNOC). Specimens were also screened for plant DNA to elucidate the sources of their sugar meals. This study provides the first data on sand fly activity and associated factors in Austria, an important transient Central European country to elucidate environmental requirements of sand flies in temperate regions. The results will contribute to detect new sand fly breeding sites and to assess their current distribution, potential expansion and epidemiological significance in Central Europe. Funding: Austrian Ministry of Defence, research stay of Edwin Kniha at Charles University was supported by Infravec2.

Ecological niche modelling of *Nyssomyia trapidoi* and *Nyssomyia ylephiletor*, vectors of *Leishmania*, with comments on their geographical distribution

Felipe Dutra Rêgo*¹, Caroline Amaral Machado¹, Paloma Helena Fernandes Shimabukuro^{1,2}

¹Grupo de Estudos em Leishmanioses, Instituto René Rachou, Fundação Oswaldo Cruz, Av. Augusto de Lima, 1715, Barro Preto, 30190-002 Belo Horizonte, MG, Brazil

²Centro de Referência Nacional e Internacional para Flebotômíneos (COLFLEB), Instituto René Rachou, Fundação Oswaldo Cruz, Av. Augusto de Lima, 1715, Barro Preto, 30190-002 Belo Horizonte, MG, Brazil.

*Corresponding author: felipedutra04@hotmail.com

Keywords: Diptera, Phlebotominae, geographical distribution, leishmaniasis, sand flies.

Leishmaniasis is a neglected, vector-borne disease occurring in the poorest countries and most vulnerable populations that has difficult access to health services. Leishmaniasis has worldwide distribution, and in America, 18 countries are endemic. In 2017, about 75% of cutaneous and mucosal cases were reported by Brazil, Colombia, Peru and Nicaragua. In this study, we evaluated the potential patterns of distribution using ecological niche modelling for of *Nyssomyia trapidoi* and *N. ylephiletor*, both vectors of *Leishmania* in northern areas of South America and in many countries of Central America. For that end, we produced a dataset with records for the distribution for both species collated from the literature and from museum specimens. In total, our database contains 313 records for *N. trapidoi* and *N. ylephiletor*. Our models were based on bioclimatic variables from Worldclim and show predicted distributions of *N. trapidoi* and *N. ylephiletor*. These two species present overlapping occurrences in countries where both are known to be incriminated/suspected vectors (Panama and Costa Rica). But also, our models showed a more northern distribution for *N. ylephiletor* from the south of Mexico to Panama, and from Ecuador to Guatemala and Belize where they were never found. Distribution maps can aid health authorities in the identification of potential risk areas for transmission of *Leishmania* and help in the organization of prevention and control programs.

VectorBase: A bioinformatics resource for invertebrate vectors and other organisms related with human diseases

Mary Ann McDowell¹ and the VectorBase Consortium, Eck Institute for Global Health, Department of Biological Sciences, University of Notre Dame

¹Department of Biological Sciences, Eck Institute for Global Health, University of Notre Dame

*Corresponding author: mcdowell.11@nd.edu

Keywords: *Phlebotomus papatasi*, *Lutzomyia longipalpis*, population genetics, genome

VectorBase.org is a free, web-based bioinformatics resource center (BRC), for invertebrate vectors of human pathogens funded by the United States National Institute for Allergy and Infectious Diseases. The database is the ‘home’ of 62 genomes of arthropod vectors and pests (e.g. *Lutzomyia longipalpis*, *Phlebotomus papatasi*, *Anopheles gambiae*, *Aedes aegypti*, and *Culex quinquefasciatus* among other species in Diptera, Hemiptera, Phthiraptera, and Acari), phylogenetically related species, and an intermediate host (the snail, *Biopmphalaria glabrata*). For most of the genomes VectorBase provided the genome wide automated annotation and currently maintains the software Apollo for gene manual annotations from the community. This database also hosts transcriptomic data (microarray and RNAseq), proteomes (mass spectrometry) and population data (single nucleotide polymorphisms, microsatellites, insecticide resistance, and other genotypes and phenotypes). Primary data is imported from external databases, directly submitted by users or generated and computed by VectorBase. The hosted data has been used for basic translational research using data in new or re-purpose analyses, descriptions, and hypothesis testing. Raw and processed data can be exported or downloaded in a variety of different formats, visualized, browsed, queried, and analyzed with VectorBase or external tools. The website has extensive documentation resources for new and experienced users, including tutorials, video tutorials, and practice exercises. In addition to live webinars on specific topics, groups can request a virtual laboratory meeting with our outreach staff. Thesis or publications using this database are kindly asked to reference the paper(s) where the data was originally published and VectorBase’s most recent paper, as explained in the website under the “Help” navigation tab. To contact us, please send a message to infor@vectorbase.org. VectorBase was one of eight BRCs originally founded in 2004 and today is one of five covering influenza virus (IRD), all other viruses (ViPR), bacteria (PATRIC), eukaryotic pathogens and fungi (EuPathDB), and invertebrate vectors of human pathogens (VectorBase). The BRC funding cycles span five years and the current contract will end September 2019. For the fourth round of BRC funding, NIAID-NIH will further consolidate the BRCs by requesting applications for only two contracts, one that covers bacteria and viruses and the other to cover protozoa, fungi and invertebrate vectors of human pathogens.

Multi scale environmental determinants of *Leishmania* vectors in urban and rural context, Argentina

MG Quintana^{1,2,3*}, MS. Santini^{2,4}, R Cavia^{2,5}, MF Martínez¹, MS Fernández^{2,6}, AA Pérez⁶, JM Direni Mancini^{2,3}, OD Salomón^{1,2}.

¹Instituto Nacional de Medicina Tropical (INMeT), ANLIS “Dr. Carlos G Malbrán”, Puerto Iguazú, Misiones, Argentina.

²Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

³Instituto Superior de Entomología “Dr. Abraham Willink”, San Miguel de Tucumán, Tucumán, Argentina.

⁴Centro Nacional de Diagnóstico e Investigación en Endemo-epidemias (CeNDIE), ANLIS “Dr. Carlos G Malbrán”, Buenos Aires, Argentina.

⁵Departamento de Ecología, Genética y Evolución, FCEN, Universidad de Buenos Aires e Instituto de Ecología, Genética y Evolución CONICET, Buenos Aires, Argentina,

⁶Grupo de Bioestadística Aplicada, Departamento de Ecología, Genética y Evolución, FCEN, Universidad de Buenos Aires

*Corresponding author: gabrieladelaquintana@gmail.com

Keywords: *Nyssomyia whitmani*, *Lutzomyia longipalpis*, Argentina.

In South America, cutaneous leishmaniasis and visceral leishmaniasis are emerging in the border area of Argentina, Brazil and Paraguay, including the localities of Puerto Iguazú and Puerto Libertad, northeast Argentina. This area reported outbreaks of cutaneous leishmaniasis since the '90s, with presence of *Nyssomyia whitmani* as the main vector related to unmodified environments and its ecotones. Regarding to visceral leishmaniasis urban reports started in 2010 with *Lutzomyia longipalpis* as the main vector. The main objective of this study was to evaluate environmental determinants that influence in the presence and abundance of main vectors of leishmaniasis. We divided into a grid of 400 m² the urban and periurban environments of both cities, with 55/15 samples sites respectively and we set 12/13 sample sites in rural areas and natural forests. The selection criterion for all sites was the ‘critical sites. One REDILA-BL trap was set for three consecutive nights, and a total of 68 meso and microscale environmental characteristics were surveyed simultaneously, and landscape environmental characteristics were evaluated using the percentage of different land cover classes estimated at 25 m, 50 m, 100 m and 250 m approximately around each sampled site. Land cover classes were defined ad-hoc and included: 1) Trees/shrubs, 2) Herbaceous, 3) Crops, 4) Bare soil, 5) Impervious or constructed surfaces and 6) Water bodies. To evaluate the association between *Ny. whitmani* and *Lu. longipalpis* abundance and environmental variables, multiple regression analysis using Generalized Linear Model with zero-inflated negative binomial distribution was used. The ‘zero-inflated’ component of the *Lu. longipalpis* absence was positively associated with the mean NDWI at 250 m radius area, while the count component was positively associated to urban services (drinking water service, garbage collection service, public sewer connection) at mesoscale level, and to the presence of blood sources (chickens, dogs or both) at micro scale level. For *Ny. whitmani*, no variable was found associated with the excess of absences, while the count component was positively associated with tree cover percentage at 50 m radius area and garbage collection service at mesoscale level, and number of hosted people and presence of chickens at micro scale. *Lutzomyia longipalpis* has urban distribution related to urbanization indicators, food presence and water bodies in the closeness. *Nyssomyia whitmani* has preference for areas with higher vegetation presence, in mixed landscapes (periurban, rural, natural areas). The scales and variables associated will allow improving surveillance strategies, prevention and control to the emergence and dispersion of VL and CL in the Argentina-Brazil-Paraguay border region.

Financial Support IDRC-Canada Project #107577-001, Ministry of Health of Argentina

Identification of vectors and possible vectors of leishmaniasis in north region of Ecuador

R. Cortez¹, E. Espinosa², M. Baquero², G. Vela S.² Zapata S.*²

¹ Colegio de Ciencias Biológicas y Ambientales, Universidad San Francisco de Quito.

² Instituto de Microbiología, Universidad San Francisco de Quito, Quito, Ecuador

*Corresponding author: szapata@usfq.edu.ec

Keywords: Sandflies, kDNA, mtDNA, pNOC, *Endotrypanum*

After the first report of cutaneous leishmaniasis in Ecuador (1920), multiple studies have been developed focused on taxonomy, fauna and ecology of the vector as well as on transmission, diagnosis and prevalence of the disease. Leishmaniasis is present 22 of the 24 provinces of Ecuador. The provinces with the highest incidence of the disease are Pichincha and Esmeraldas. The objective of the present study was to determine the presence of kinetoplast DNA (kDNA) by real-time PCR in vectors and possible vectors of *Leishmania* in 5 provinces: Esmeraldas, Bolívar, Manabí, Pichincha and Orellana. During 2013 and 2014 a total of 2333 specimens were collected and analyzed. The rate of infection with kDNA was 11.15% and corresponded to the genus *Endotrypanum*. The infection rate of *Leishmania* was 0.67%. It is reported for the first time to *Psychodopygus carrerai thula* and *Pintomyia serrana* infected with DNA of *Leishmania guyanensis*, the same ones that in Ecuador are not yet reported as *Leishmania* vectors. The blood origin of the specimens under study corresponded to 9 vertebrate species *Homo sapiens*, *Choloepus hoffmanni*, *Bos taurus*, *Pecari tajacu*, *Capra hircus*, *Equus caballus*, *Cebus capucinus*, *Tapirus terrestris* and *Potos flavus*; distributed around the 5 provinces analyzed. The monitoring of the presence of kDNA in populations of sandflies in endemic areas could be crucial to understanding the dynamics of transmission of the parasite and the identification of possible new vectors of leishmaniasis.

Evaluation of a pan-*Leishmania* SL-RNA qPCR assay for parasite detection in laboratory and field collected sand flies.

Myrthe Pareyn^{*1}, Rik Hendrickx², Sarah Hendrickx², Natalie Van Houtte¹, Nigatu Girma³, Gert Van der Auwera⁴, Herwig Leirs¹, Guy Caljon²

¹ Biology department, University of Antwerp, Wilrijk, Belgium

² Laboratory of Microbiology, Parasitology and Hygiene, University of Antwerp, Wilrijk, Belgium

³ Biology department, Arba Minch University, Arba Minch, Ethiopia 4 Biomedical sciences department, Institute of Tropical Medicine, Antwerp, Belgium

*Corresponding author: myrthe.pareyn@uantwerpen.be

Keywords: SL-RNA qPCR, sand flies, Ethiopia

In order to screen field collected sand flies for the presence of *Leishmania*, a large sample size is required, as the prevalence is generally low, even in endemic areas. The golden standard for parasite detection in sand flies, microscopic examination, is very time consuming, currently resulting in a shift towards molecular methods. A variety of (real-time) PCR methods targeting different gene fragments has been described, many of which remain to be validated on multiple *Leishmania* species in different tissues, or have issues regarding quantification. Recently, a pan-*Leishmania* SYBR Green qPCR assay was developed, targeting the conserved 39bp spliced-leader (SL) mini-exon RNA sequence, which shows excellent sensitivity and specificity. The assay was able to detect eight Old and New world *Leishmania* species with equal threshold cycle values and with a very low limit of detection. The assay was validated on tissue samples of infected hamsters, promastigote spiked human blood and blood nucleic acid extracts from visceral leishmaniasis patient blood samples. This study aimed to determine the limit of detection (LoD) and quantification (LoQ) of the SL-RNA qPCR assay on laboratory infected and field collected sand flies. Laboratory *Lutzomyia longipalpis* sand flies were spiked with a serial dilution of *L. major* promastigotes. Nucleic acids were extracted with a high-salt extraction buffer and samples were subjected to qPCR assays targeting (i) SL-RNA, (ii) kDNA/18S DNA in a multiplex Taqman probe assay and (iii) kDNA in a SYBR Green assay with an alternate set of primers. Standard curves were generated, from which the theoretical LoD and LoQ were determined. Additionally, *L. longipalpis* were infected by artificial feeding on *L. major* spiked blood and subjected to parasite quantification by either microscopic analysis or the various molecular assays. The specimen with the highest estimated parasite content was subjected to a serial dilution to generate standard curves and determine the LoD and LoQ. Furthermore, field collected Ethiopian *Phlebotomus pedifer* sand flies infected with *L. aethiopica*, preserved and extracted under unfavorable procedures for downstream RNA detection, were used to comparatively assess the suitability of the various molecular assays for field application. This study revealed that the pan-*Leishmania* qPCR is a very promising tool for parasite detection and quantification in field and laboratory infected sand flies, even if specimens are preserved under suboptimal conditions and following a crude nucleic acid extraction procedure.

List of Participants

Name	Country	e-mail
Agathe Chavy	France	agathe.chavy@hotmail.fr
Alessandra Gutierrez de Oliveira	Brazil	alessandra.gutierrez.oliveira@gmail.com
Alongkot Ponlawat	Thailand	Alongkotp.fsn@afirms.org
Andrei Mihalca	Romania	amihalca@usamvcluj.ro
Andreia Fernandes Brilhante	Brazil	brilhanteaf@usp.br
Andrey Jose De Andrade	Brazil	bioandrey@gmail.com
Barbora Vojtkova	Czech Republic	vojtkob@natur.cuni.cz
Blima Fux	Brazil	blimafux@yahoo.com.br
Carina Margonari de Souza	Brazil	margonari@minas.fiocruz.br
Carla Maia	Portugal	carlamaia@ihmt.unl.pt
Cristina Daniela Pop (Cazan)	Romania	cristina.cazan@usamvcluj.ro
Elyes Zhioua	Tunisia	elyes.zhioua@gmail.com
Emma Howson	United Kingdom	emma.howson@pirbright.ac.uk
Eunice Aparecida Bianchi Galati	Brazil	egalati@usp.br
Everton Falcão de Oliveira	Brazil	efalcao.oliveira@gmail.com
Fano José Randrianambinintsoa	France	fano-jose.randrianambinintsoa1@univ-reims.fr
Fatima Amaro	Portugal	fatima.amaro@insa.min-saude.pt
Felipe Dutra Rêgo	Brazil	felipedutra04@hotmail.com
Flávia Benini da Rocha Silva	Brazil	flavinhabenini@gmail.com
Frankiln Espinosa	Ecuador	espinosamfa@gmail.com
Fredy Galvis Ovallos	Brazil	galvisfregao@gmail.com
Guy Caljon	Belgium	Guy.Caljon@uantwerpen.be
Herintha Coeto Neitzke-Abreu	Brazil	herintha@yahoo.com.br
Horacio Cadena Peña	Colombia	horacioopcadena@gmail.com
Idris Alabi	Mexico	alabis999@yahoo.com
Ivan Dario Velez	Colombia	idvelez@pecet-colombia.org
Jan Votypka	Czech Republic	jan.votypka@natur.cuni.cz
Jerome Depaquit	France	jerome.depaquit@univ-reims.fr
José Dilermando Andrade Filho	Brazil	jandrade@minas.fiocruz.br
Jovana Sadlova	Czech Republic	jovana.sadlova@natur.cuni.cz
Juan Pablo Ochoa	Argentina	jpchoa79@hotmail.com
Juan Ramón Rosa	Argentina	juan_rosa05@yahoo.com.ar
Karina Bertazo Del Carro Giacomini	Brazil	karina_bertazo@hotmail.com
Lenea Campino	Portugal	carlamaia@ihmt.unl.pt
Luigi Gradoni	Italy	luigi.gradoni@iss.it
Maes Louis	Belgium	louis.maes@uantwerpen.be
Major S. Dhillon	USA	MDHILLON@NORTHWESTMVCD.ORG

Mara Cristina Pinto	Brazil	marap@fcfar.unesp.br
Marcelo Ortigao	USA	jose-marcelo.ramalho-ortigao@usuhs.edu
Márcia Moreira de Ávila	Portugal	marcia.avila@ifac.edu.br
María Gabriela Quintana	Argentina	gabrieladelaquintana@gmail.com
María Lucrecia Villarquide	Argentina	mlucrecia.v@gmail.com
Mariza Fordellone Rosa Cruz	Brazil	mfordellone@uenp.edu.br
Mary Ann McDowell	United Kingdom	mcdowell.11@nd.edu
Mary Cameron	United Kingdom	mary.cameron@lshtm.ac.uk
Mathieu Loyer	France	reyol.cobalt@gmail.com
Mattia Calzolari	Italy	mattia.calzolari@izsler.it
Merat Melanie	France	melanie.merat@laposte.net
Michal Cikrt	Czech Republic	Michal.Cikrt@seznam.cz
Myrthe Pareyn	Belgium	myrthe.pareyn@uantwerpen.be
Nagila Francinete Costa Secundino	Brazil	secundinon@gmail.com
Nazli Ayhan Yilmaz	France	nazliayhann@gmail.com
Nil Rahola	France	nil.rahola@ird.fr
Nilsa González Brítez	Paraguay	gbritez.nilsa@gmail.com
Ognyan Mikov	Bulgaria	mikov@ncipd.org
Oscar Daniel Salomón	Argentina	odanielsalomon@gmail.com
Ozge Erisoz Kasap	Turkey	ozgeerisoz@yahoo.com
Paola Valeria Yachuk	Argentina	hmsfurious@hotmail.com
Petr Volf	Czech Republic	volf@cesnet.cz
Petra Cikrtova	Czech Republic	sumovap@natur.cuni.cz
Prevot Ghislaine	France	ghislaine.prevot@univ-guyane.fr
Jorian Prudhomme	France	jorian.prudhomme@hotmail.fr
Rafaella Albuquerque e Silva	Brazil	rafaella.silva@saude.gov.br
Renato León	Ecuador	rleon@usfq.edu.ec
Rodrigo Pedro Pinto Soares	Brazil	rsoares@minas.fiocruz.br
Rosa Galvez	Spain	rosa.galvez@uam.es
Sandra Inés Enríquez Morillo	Ecuador	ienriquez@uce.edu.ec
Shaden Kamhawi	USA	skamhawi@niaid.nih.gov
Shannon McIntyre	United Kingdom	shannon.mcintyre1@lshtm.ac.uk
Sofía Lorián Moya	Argentina	sofialorian@gmail.com
Sonia Zapata Mena	Ecuador	szapata@usfq.edu.ec
Tereza Lestinova	Czech Republic	terka.kratochvilova@seznam.cz
Thais Lemos da Silva	Brazil	thais.silva@ioc.fiocruz.br
Tomas Becvar	Czech Republic	becvart@natur.cuni.cz
Ulrich Bernier	USA	UBERNIER@COX.NET
Vera Margarete Scarpassa	Brazil	vera@inpa.gov.br
Vicente Estevam Machado	Brazil	vicentemachado@yahoo.com.br
Vit Dvorak	Czech Republic	icejumper@seznam.cz

Vladimir Iovic
Wagner De Souza Fernandes
Yester Basmadjian

Slovenia
Brazil
Uruguay

vladimir.ivic@famnit.upr.si
wagner.ufms@gmail.com
yesterb@gmail.com