RT.01 – Immunity against protozoans

RT.01-01 - **Exhaustion on Leishmania amazonensis and *Leishmania braziliensis* infection**

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Chronic antigenic stimulation can lead to the induction of T cell exhaustion, which hampers the T cell response necessary for controlling the infectious process. In the case of infection with Leishmania amazonensis in mice, a suppressive capacity is observed, wherein the expression of PD-1 on lymphocytes and PD-L1 on macrophages, neutrophils, and dendritic cells contributes to the failure in generating an effective immune response. The infection by L. amazonensis triggers the expression of PD-L1 on these cells, indicating that the parasite exploits this pathway to evade the immune response. Treatment with anti-PD1 or anti-PD-L1 antibodies can control the parasite load by reinvigorating the CD4 + and CD8 + T cell responses and promoting increased production of interferon-gamma. On the other hand, infection with L. braziliensis exhibits the ability to induce an effective immune response capable of controlling the parasite load. However, when analyzing the infection by L. braziliensis, it is also observed that the parasite induces PD-L1 expression both in vitro and in vivo. Treatment with anti-PD1 and anti-PD-L1 antibodies in vivo enhances the control of the parasite load, indicating that the exhaustion process also occurs in Leishmania braziliensis infection. Furthermore, immunotherapy has the potential to improve the ability to control the parasite load by enhancing the interferon-gamma response. These findings support previous data obtained from patients with cutaneous leishmaniasis caused by Leishmania braziliensis and suggest the possibility of using immunotherapy in patients who do not respond well to conventional treatments.**Keywords**:Leishmania amazonensis;Leishmania braziliensis;exhaustion.

RT.01-02 - **Heme trafficking at the Leishmania-host interface**

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Parasites develop adaptations that allow them to acquire nutrients unidirectionally from their host to sustain their growth and reproduction. One such adaptation is the uptake of heme, an iron-containing organic ring that certain parasites cannot produce but is synthesized by all vertebrate hosts by a highly conserved multi-step pathway. This requirement for external heme is found in both parasitic nematodes and single-celled parasites, i.e, Trypanosoma and Leishmania. Thus drugs that target heme transport pathways unique to the parasite and not shared by its mammalian host have interesting therapeutic potential. We discovered HRG1, the first eukaryotic heme importer. HRG1 homologs exist across species but are genetically divergent, with only 13% identity between human HRG1 and LHR1, its homolog in Leishmania. We propose that Leishmania growth and virulence is inextricably intertwined with host heme and iron status as Leishmania is a heme auxotroph and resides within macrophages, the very cell that recycles body heme-iron. To study the relationship
between host heme status and parasitemia, we employ genetically-altered mouse infection models, ex vivo cell cultures, and functional assays in yeast. Results and implications from these studies will be discussed. Uncovering the role of essential nutrients on leishmaniasis could elucidate potential parasite-specific therapeutic targets that would limit host toxicity and improve quality of life.**Keywords:**Heme;Iron;Anemia.

RT.01-03 - **Regulation of Immunological Memory to *Toxoplasma gondii***

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Tallied up, parasites cause 96 million Disability Adjusted Life Years (DALYs) and 2 billion new infections every year. And yet, a fully protective vaccine to prevent any parasitic disease has eluded immunologists and parasitologists alike. Addressing why immunological memory fails and how vaccination can be used to prevent parasitic disease underpins the research in the Jensen lab. Specifically, we study requirements for heterologous immunity to highly virulent strains of the widespread intracellular parasite of animals, *Toxoplasma gondii*. We have identified parasite virulence factors that contribute to immune evasion and found that host genetics are major determinants of acquired immunity. In this seminar, I would like to discuss two genetic mapping experiments designed to understand mechanisms of acquired immunity to *T. gondii* that follow natural infection or vaccination. First, results from a large Collaborative Cross (CC) recombinant inbred mouse panel, which incorporates alleles from three sub-species of *Mus musculus*, revealed a significant contribution from a single locus centered on the Wnt-signaling pathway transcription factor, *Tcf7* or TCF-1. In resistant mice, enhanced central memory CD8 T cell responses and TCF-1 expression underlie the success of attenuated *T. gondii* vaccines against highly virulent South American strains. Second, results from a recombinant inbred (AXB;BXA) mouse panel revealed an unexpected role for B-1 cells and a regulator of NF-κB, *Nfkbid* or IκBNS, as paramount for humoral immunity to this parasite. Recently, we have found that antigens targeted by antibodies appear highly sensitive to the GPI-anchor, and when the GPI is modified in *T. gondii,* it significantly alters parasite virulence. In summary, we reason vaccination strategies aimed at *T. gondii* prevention, and likely other parasites, should target signaling pathways that activate the above transcriptional regulators to elicit appropriate immunological memory responses. Choice of immunogens will also be critical. Perhaps by including conserved biomolecules like the GPI, which is both an antigen and TLR agonist, heterologous immunity can be achieved against multiple parasite strains encountered in nature. **Supported by::** NIH **Keywords:**Forward Genetics;Vaccines;Toxoplasma gondii.

RT.01-04 - ***Trypanosoma cruzi* undermines the host immune response through purinergic signaling activation**

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Chagas disease, also known as American trypanosomiasis, is the primary chronic infectious heart disease worldwide and is caused by the intracellular parasite Trypanosoma cruzi. Following infection, both innate and adaptive responses control circulating parasite levels, but are insufficient to completely clear the infection. Therefore, one of the main challenges in understanding Chagas disease immunopathology is to find out why the parasite is not completely cleared, being able to sustain a pathological inflammatory environment that underlies the progression of cardiac lesions. After infection, the influx of immune cells consumes large amounts of oxygen, and ischemic cells rapidly respond to the hypoxic and inflammatory environment by releasing ATP. This extracellular ATP (eATP) triggers microbicidal immune responses but is quickly hydrolyzed to the potent immunosuppressive metabolite adenosine, mainly via the concerted activity of CD39 and CD73 ectoenzymes. We propose that CD73-derived adenosine has a central role in regulating immune response to T. cruzi infection. We have reported that pharmacological inhibition of CD73 activity early during the acute phase ameliorates the outcome of murine Chagas cardiomyopathy by preventing the shift of cardiac type-M1 to anti-inflammatory M2 macrophage that occurs at 7 days postinfection (dpi), enhancing nitric oxide (NO) production, and diminishing the frequency of IL-4- and

IL-10- producing CD4+ T-cells. In concert, CD73 abrogation decreased the local parasite burden and improved cardiac functionality. In accord, predominant presence of T-cells in Chagas patient myocardium that correlated with the number of CD73-expressing cells and the presence of HIF-1α+ cells were also determined. In contrast, circulating T-lymphocytes display reduced expression of CD39 and CD73 ectoenzymes in chronically infected individuals, associated with increased plasmatic ATP levels compared with non-infected donors. Thus, it is plausible that in the periphery, T-cells fail to promote eATP degradation and an inflammatory microenvironment, but in the myocardium, T-lymphocytes upregulate the enzymatic machinery to enhance ATP metabolization fostering a regulatory milieu. The results evidence that T. cruzi subverts the immune response to infection through purinergic signaling activation in experimental and human Chagas disease. **Supported by::** R01 (RAI176457A); SECyT-UNC; Agencia I+D+i-FONCyT; CONICET
Keywords: Chronic Chagas cardiomyopathy;lymphocytes;CD73; adenosine.

RT.02 – Protozoan Vectors and Viruses

RT.02-01 - **Identification and characterization of new viruses in *Rhodnius prolixus***

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Hemimetabolous insects of the subfamily Triatominae account for more than 150 species, including *Rhodnius prolixus* and *Triatoma infestans*, two hematophagous species firmly associated with the transmission of Chagas disease. The etiologic agent of the parasitosis is the protozoan *Trypanosoma cruzi*, which is transmitted to the human host during the blood meal. Despite the medical relevance, the range of viral species infecting Triatomine insects remains largely unexplored. In stark contrast with mosquitos and other insects,where the host/virus interaction has been investigated in much detail, our knowledge of triatomine viruses mostly relies on the characterization of Triatoma virus (TrV). TrV proved to be a triatomine-specific entomopathogenic virus causing leg paralysis and death in triatomine colonies. Very recently, we identified and partially characterized seven new viruses in Rhodnius prolixus using metatranscriptomic approaches. *Rhodnius prolixus* viruses 1-7 (RpV1-7) can be classified in three different families: Iflaviridae (RpV1 and 2), Permutotetraviridae (RpV3, 4 and 7) and Solemoviridae (RpV5 and 6). Interestingly, the putative RpV2 polyprotein shares a high degree of aminoacid sequence identity with that of Slow Bee Paralysis Virus, which is responsible for the collapse of bee hives and huge economic losses. All the viruses display single-stranded positive sense RNA genomes and are maintained in the insect population via transovarial vertical transmission and coprophagy. Different from TrV however, the RpVs do not show apparent effects neither on the viability nor on the fertility of the insects. We found that an antiviral system centered on viral small interfering RNAs is active in Rhodnius and might explain at least in part these findings. Finally, we asked whether the RpVs can infect *Trypanosoma cruzi*. In a recent study, it was shown that enveloped and non-enveloped viral-like particles can be observed in the cytoplasm of *T. cruzi* by electron microscopy. In agreement with these data, we found that the RpVs can infect T. cruzi and, at least RpV1, is detectable in the cell cytoplasm. Furthermore, our electron microscopy assays reveal viral-like particles in Rhodnius'; gut, thus suggesting that *T. cruzi* can be infected in nature while passing through the host intestine. Our results start to shed light on the complexity of the triatomine virome and its ternary interaction with the insect and the protozoan *T. cruzi*.
Keywords: Rhodnius;virus;Trypanosoma.

RT.02-02 - **Identification of endogenous Viral elements (EVEs) in embryonic cell lines and insects of *L. longipalpis* and others phlebotomine sandflies from Brazil**

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The phlebotomines of the genera *Phlebotomus* and *Lutzomyia* are the main vectors of leishmaniasis in the Old and New World, respectively. In addition to vectors of leishmaniasis, the phlebotomines of the Old World are known to be vectors of several arboviruses. Little is known about the vectorial capacity of New World sandflies in the transmission of viruses to humans. Our group has conducted various studies with Lutzomyia longipalpis, Brazil's main vector of visceral leishmaniasis. One of the main models used for *L. longipalpis in vitro* studies is the LL-5 embryonic cell line. These cells, when transfected with double-stranded RNAs, show non-specific antiviral activity and the conditioned medium of these cells induces this antiviral phenotype in non-transfected cells. Transcriptomic analysis of this conditioned medium revealed the presence of RNAs that encode for different Rhabdovirus proteins. Rhabdoviruses are RNA viruses, so it these sequences were expected to be identified in cDNA samples from LL-5 cells and, potentially, in samples of adult insects. DNA samples were also used in PCRs as a negative control since these viruses never appear in the form of DNA during their replicative cycle. Surprisingly, these elements were also identified in these DNA samples, indicating insertion into the genome. The presence of these endogenous viral elements (EVEs) was confirmed by *in silico* and *in vitro* studies.
The presence of EVEs sequences integrated into the genome of vertebrates, invertebrates, and plants is described in various works. Depending on the role they play in the host along time EVEs, can be eliminated or persist during the evolutionary process. Some EVEs may exert beneficial functions for their hosts in a process known as exaptation. A classic example the transcription and/or translation of these EVEs by the host itself leading to resistance to viral infections.
In this project, we also investigated the presence of these inserts in different sand flies populations in Brazil. **Supported by::** CNPq, Faperj **Keywords:**Endogenous Viral Element;Lutzomyia longipalpis ;RNA.

RT.02-03 - **Insights into the Interaction between protozoan parasite vectors and Viruses**

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Studying viral agents within vectors such as Rhodnius and Lutzomyia holds paramount significance due to their profound implications for public health and the transmission dynamics of diseases. These arthropod vectors assume pivotal roles in disseminating parasitic infections, such as Chagas disease and leishmaniasis, which exert a substantial global burden. Elucidating the virological infections within these vectors offers insights into potential co-infection scenarios and their synergistic ramifications on disease manifestations. Furthermore, delving into viral interactions provides opportunities to advance targeted control methodologies that can curtail vector populations and disrupt disease transmission cycles. Thus, we employed advanced small RNA deep sequencing techniques on specimens derived from Lutzomyia longipalpis and Rhodnius prolixus. Our primary objective was to discern the presence of viral entities and their plausible interactions with the insect immune systems. In the context of Lutzomyia longipalpis, our investigations led to the identification of six novel viruses. These viruses exhibited diverse profiles of virus-derived small RNA, with some indicative of RNA interference (RNAi) pathways targeting, while others suggested potential RNA degradation patterns possibly resulting from accumulation, implying inhibition of the siRNA pathway. Notably, we examined the non-coding small RNA response of L. longipalpis following artificial infections with Vesicular stomatitis virus (VSV), a virus naturally

propagated by this phlebotomine in the wild. This analysis unveiled a heightened production of VSV-derived siRNAs, accompanied by the modulation of host-derived miRNAs. Additionally, virus-derived small RNAs exhibited different profiles for specific species. Moreover, leveraging small RNAs aligned to putative viral sequences enabled successful discrimination between endogenous and exogenous elements. For Rhodnius, a similar approach involving deep small RNA sequencing disclosed the presence of new viruses spanning at least five families: Permutotetraviridae, Iflaviridae, Sobemoviridae, Narnaviridae, and Partitiviridae. Through an oxidation-based strategy, we investigated the association of virus-derived small RNAs with Argonaute proteins. In both Rhodnius and Lutzomyia cases, a robust small RNA-mediated response against viral agents was evident, implying that the RNAi pathway is involved in the immune response of these protozoan parasite vectors against viral infections.

In conclusion, the comprehensive exploration of viral agents and their interplay with antiviral pathways within these vectors stands as a pivotal stride towards enhancing our capacity to mitigate the repercussions of vector-borne illnesses. This endeavor safeguards human and animal health while fostering the development of more efficacious and sustainable interventions within the realm of public health.

Keywords: Virus infection, protozoan parasite vectors;Lutzomyia;Rhodnius; RNAi pathway.

RT.03 – Fascinating peculiarities regarding protozoan parasites

RT.03-01 - **Uncovering the Mechanisms of VSG mRNA Regulation through Multi-Omics Analysis**

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Trypanosoma brucei causes sleeping sickness in humans and nagana in cattle. The unicellular parasite is transmitted by the bloodsucking tsetse fly. In the mammalian host's circulation, the parasite avoids the host immune response by periodically replacing a monolayer of variant surface glycoproteins (VSG) that covers its cell surface. Such antigenic variation is a key pathogenesis mechanism that enables *T. brucei* to establish long-term infections. VSG is expressed exclusively from subtelomere loci in a strictly monoallelic manner, and DNA recombination is an important VSG switching pathway. VSG mRNA accounts for nearly 10% of total mRNA, and its high stability is essential for trypanosome survival. To determine the mechanism by which VSG mRNA stability is maintained, we used mRNA affinity purification to identify the proteins specifically associated with the VSG mRNP. By purifying the VSG mRNA, we have recently identified Cyclin F-Box 2 (CFB2) as the protein responsible for VSG mRNA stability in bloodstream forms. As occurs in the active VSG KD, we observed that depletion of CFB2 leads to decreased levels of active VSG transcripts and a cytokinesis arrest. CFB2 recognizes a conserved 16mer element that is found in the 3'-UTRs of all VSG mRNAs. Moreover, we could demonstrate the mechanism by which CFB2 acts: recruitment of a stabilizing complex that includes MKT1, PBP1, PABP2, and the cap-binding translation initiation complex EIF4E6/G5. We have now validated additional candidates from this VSG mRNA-bound proteome and identified a novel protein as a regulator of the VSG switching pathway. RNA-seq analysis indicated that this novel protein is important in maintaining the level of active VSG mRNA, similarly to the CFB2 depletion. But unlike the CFB2 KD, its depletion did not induce a cytokinesis arrest. Surprisingly, its depletion caused a rapid increase in the levels of phosphorylated H2A and replication protein A1 (RPA1), proteins implicated in DNA double-strand break (DSB) repair. More notably, this led to a dramatic increment in the VSG switching rate (~150-fold up) with switchers arising predominantly by recombination processes. Reflecting this phenotype, we named this gene 'Suppressor of VSG Switching 1 (SVS1)'. Our findings suggest the existence of a potential interaction

between VSG mRNA surveillance and VSG switching in this parasite. We are currently investigating the mechanisms underlying the role of SVS1 to gain a deeper understanding of how VSG switching and the regulation of monoallelic VSG expression are controlled. Beyond *T. brucei*, the mRNP purification approach has the potential to supply detailed biological insight into metabolism of relatively abundant mRNAs in any eukaryote organism. **Supported by**:: NIH, AGENCIA I+D+i
Keywords:VSG;mRNA stability;antigenic variation.

RT.03-02 - **The chromatin and protein acetylation in *Toxoplasma gondii***

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Toxoplasma gondii is an obligate intracellular parasite and the causative agent of toxoplasmosis. In Brazil, this disease can reach 60% of incidence, depending on the region, culture, and socioeconomic conditions. Although usually asymptomatic, toxoplasmosis is extremely dangerous when acquired during pregnancy or in immunocompromised patients and can result in severe damage. In Brazil, it is one of the main causes of severe eye damage, including blindness. In Brazil, the disease shows more severe symptoms than those observed in the northern hemisphere, which indicates that the strains found in our territory are more virulent when compared to those found in the USA and Europe. The parasite's life cycle passes through sexual stages in felines and asexual stages in intermediate hosts, including humans. These transitions require fine control of gene expression. At the center of this regulation are the histones. Toxoplasma has four core histones, with high similarity with human histones. However, the presence of a histone linker H1 was still a question. We identified a small and basic protein (TgH1-like) that forms a complex with most histones. The knockout of tgh1-like resulted in fewer peripheral heterochromatin and unexpected asynchronous division. Histones are subject to posttranslational modifications, and according to ToxoDB, TgH1-like has phosphorylation and ubiquitylation sites, which were mutated and confirmed the problem during division, showing those modifications are essential for the correct cell cycle. As histone, H1-like, all histones, and other proteins are targets for several post-translation modifications. One of the most abundant is acetylation which can affect chromatin compaction and other functions. Toxoplasma has five classic HDACs, and we started characterizing those with unique characteristics: TgHDAC2 and TgHADC4. TgHDAC2 has around 200 amino acids inside the HDAC domain, specific to some members of the *Apicomplexa phylum*. The knockout of tghdac2 showed defects in virulence and replication. Structure analyses suggested that these unique sequences are more related to function than stability. The location of TgHDAC2 surrounds the daughter cells. TgHDAC4, a class IV histone deacetylase, is the most understandable class of deacetylases. By phylogenetic analysis, we observed that TgHDAC4 group with prokaryotic HDACs. TgHDAC4 is located in the apicoplast, a plastid-like structure with secondary endosymbiotic origin. TgHDAC4 is immunoprecipitated with proteins in the apicoplast and apparently is unrelated to the apicoplast's DNA. Several attempts to delete the protein suggest it is essential. We currently use the TeT system to analyze the TgHDAC4 function by condition KO. HDACs are known and proven inhibitor targets for several diseases. Understanding how Toxoplasma's HDACs work could result in a potential target for better treatments for toxoplasmosis. **Keywords:**Toxoplasma;histone;histone deacetylases.

RT.03-03 - **Synthesis and translation of selenocysteine in Kinetoplastida and Bacteria**

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The genetic code, composed of 64 different codons and called “Universal”, codes for 20 amino acids. This number has already been modified to include two new amino acids, selenocysteine and pyrrolysine, using the same repertoire of 64 codons. Selenium, in particular, exists naturally in organic forms such as selenomethionine and selenocysteine and in various inorganic forms such as selenites, selenates and others. Selenium, instead of sulfur, in the form of the amino acid selenocysteine (Sec-U) results in increased catalytic activity of selenoenzymes as a result of Se being more nucleophilic and being ionized at physiological pH. The selenocysteine synthesis pathway and the respective genes for SELB, SELD, PSTK, SecSepS and tRNASerSec (SelC) have been identified in protozoa. The functional and structural characterization of the enzymes involved in the synthesis of selenocysteine, as well as the investigation of the physiological function of this pathway in protozoa, parasites or not, are the objectives that may contribute to the identification of new molecular targets and in the elucidation of the way in which the organisms evolve. recognition mechanisms between tRNA and proteins. **Keywords**: Selenocysteine;Translation;Kinetoplastida.

RT.03-04 - **Total parasite biomass but not peripheral parasitaemia is associated with endothelial and haematological perturbations in Plasmodium vivax patients**

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Plasmodium vivax is the major cause of human malaria in the Americas. How *P. vivax* infection can lead to poor clinical outcomes, despite low peripheral parasitaemia remains a matter of intense debate. Estimation of total *P. vivax* biomass based on circulating markers indicates existence of a predominant parasite population outside of circulation. In this study we investigate associations between both peripheral and total parasite biomass and host response in vivax malaria. We analysed parasite and host signatures in a cohort of uncomplicated vivax malaria patients from Manaus, Brazil, combining clinical and parasite parameters, multiplexed analysis of host responses and *ex vivo* assays. Patterns of clinical features, parasite burden and host signatures measured in plasma across the patient cohort were highly heterogeneous. Further data deconvolution revealed two patient clusters, here termed Vivax^{low} and Vivax^{high}. These patient subgroups were defined based on differences in total parasite biomass but not peripheral parasitaemia. Overall Vivax^{low} patients clustered with healthy donors and Vivax^{high} patients showed more profound alterations in haematological parameters, endothelial cell (EC) activation and glycocalyx breakdown and levels of cytokines regulating different haematopoiesis pathways compared to Vivax^{low}. Vivax^{high} patients presented more severe thrombocytopenia and lymphopenia, along with enrichment of neutrophils in the peripheral blood and increased neutrophil-to-lymphocyte ratio (NLCR). When patients’ signatures were combined, high association of total parasite biomass with a subset of markers of EC activation, thrombocytopenia and lymphopenia severity was observed. Finally, machine learning models defined a combination of host parameters measured in the circulation that could predict the extent of parasite infection outside of circulation**.** Altogether, our data show that total parasite biomass is a better predictor of perturbations in host homeostasis in *P. vivax* patients than peripheral parasitaemia.

This supports the emerging paradigm of a *P. vivax* tissue reservoir, in particular in the hematopoietic niche of bone marrow and spleen.**Keywords**:Plasmodium vivax, malaria parasite;total biomass, tissue infection;endothelial activation, haematopoiesis.

RT.04 – Current Topics in Protozoology
(organized by the Early Career Research (ECR) committee)

RT.04-01 - **Modeling of epidemiological parameters in vector-borne diseases**

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Introduction. Vectorial transmission of *Trypanosoma cruzi* by *Triatoma infestans* was eliminated from Brazil. However, the vertical mode of transmission, congenitally through a carrier mother to her baby, is the disease's main mechanism of persistence. The absence of a specific surveillance data system determines that prevalence and congenital transmission rates remain unknown. This makes harder to establish public policies and actions for the control and elimination. This study aimed to estimate the epidemiological parameters of Chagas disease in municipalities in Brazil. **Methods**. A meta-analysis was carried out to extract data on prevalence in specific population groups, in municipalities in Brazil, 2010–2022. Indicators available in the information systems were selected at municipal scale. Statistical modeling of extracted data from meta-analysis as a function of those obtained from information systems was applied using maximum likelihood and the principle of parsimony. **Results**. The 5 best models were selected, from a total of 549 tested models, to obtain a consensus model (adjusted R^2=54%). The most important predictor was from the primary care information system. The mean prevalence of the disease was estimated at 3.4% (±2.9%) in 3,662 municipalities in Brazil. Number of Chagas disease carriers was estimated in the general population (~3.9 million), women (~2.2 million) and women of childbearing age (~630 thousand). The calculated disease reproduction rate was 1.035. All estimates refer to 2015–2016. **Conclusions**. Estimated prevalence of Chagas disease in women of childbearing age is presented as an indirect estimate of the risk of vertical transmission of Chagas disease by municipality in Brazil. The estimated values are in line with those predicted by PAHO/WHO and mathematical projections. It is proposed to use this estimate in reference municipalities for a pilot project of the national pact for the elimination of congenital Chagas disease. **Supported by**:: São Paulo Research Foundation (FAPESP) grant number 21/06669-6 **Keywords:**Pregnant Women;Vertical Transmission of Infectious Diseases;Trypanosoma cruzi.

RT.04-02 - **Integrative systems biology of host-Plasmodium interactions**

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Although the burden of malaria has decreased in the last decades, the disease still affects millions of individuals and causes thousands of deaths worldwide. The factors that drive protection or pathology after infection with *Plasmodium* are still not completely solved. Systems biology emerged from changes in scientific philosophy moving from a reductionist point of view towards a holistic understanding of the whole biological systems under assessment. High-throughput technologies coupled to rapid development of computational biology spurred studies about molecular profiles of patients, controlled human malaria infection, and non-human primate models. The first

studies using these technologies have been reported almost over two decades, but limited sample sizes, lack of generalizability and requirement for robust and independent validation prevent the translational potential of findings from individual studies. We sought to use an integrative systems biology approach to analyze data from diverse and distinct cohorts and experimental models to gain novel insights into the molecular mechanisms of host-*Plasmodium* interactions. We used these data in three different studies which will be summarized here. The first study aimed to identify conserved transcriptional signatures of individuals with malaria. For that, we integrated gene expression data obtained through microarray of RNA sequencing technologies from over 800 samples. Samples were split into discovery and validation cohorts. We found a 16-gene signature composed of molecules involved in host defense (*C1QA*, *GBP1*) and metabolism (*SMPDL3A*), denominated the Malaria Meta-Signature (MMS). ROC analysis revealed that the MMS discriminates individuals with malaria from healthy controls with high-performance in discovery (AUC = 0.98) and validation (AUC = 0.97) cohorts. The MMS also distinguishes from asymptomatic *Plasmodium* infection and other febrile and inflammatory diseases. The MMS correlates with parasitemia, RBC counts and IL-1RA, IL-6, IL-10, CXC10, CCL4 in the plasma. We validated the functional of *SMPDL3A* by integration with metabolomics data, which revealed a positive correlation between *SMPDL3A* expression and levels of adenosine monophosphate (AMP) in the plasma. These findings demonstrate a robust transcriptional biomarker signature of malaria and suggest the activity of unknown metabolic regulators of the inflammatory response. In a following study, we compiled 9 datasets of dual RNA sequencing data from both malaria patients and *P. falciparum* (Pf) to identify robust associations between the transcriptional response of hosts and parasites. We reduced the dimension of data from human gene expression to 346 blood transcription modules (BTM) that reflect immune and metabolic processes in the blood. We evaluated the correlations between BTMs and Pf genes with Spearman correlation and retained associations with $p < 0.001$. Associations found in approximately 70% of the datasets (6 datasets) formed a network with 60 nodes (BTMs and Pf genes) linked by 30 edges. For example, we found interactions between RIG-I-like receptor signaling and Pf genes such as *RESA*, *KIC6,* and *HMGB3*. BTMs reflecting TLR and inflammatory signaling as well as CCR1, 7 and cell signaling we also associated with Pf *HMGB3* expression. These findings demonstrate unknown concerted activity between host and Pf gene expression and suggest novel molecules involved in innate immune activation. In a third study, we collected multiple datasets of plasma metabolomics from individuals with malaria and healthy controls. We performed an integrated analysis that revealed metabolic pathways that are commonly disrupted with malaria for independent and diverse cohorts. Taken together these studies demonstrate that multi-omics data repurposing offers effective opportunity to discover molecular features of host-*Plasmodium* interactions**.** **Supported by**:: Instituto Serrapilheira, FUNAPE, CNPq **Keywords**:Malaria;Systems Biology;Multi-omics technologies.

RT.04-03 - **Unraveling the mechanisms of endocytosis by intracellular amastigotes of *Trypanosoma cruzi***

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Endocytosis is a vital cellular process that involves the uptake of molecules and particles from the extracellular environment. It also plays a significant role in removing plasma membrane components and modulating cell signaling. In *Trypanosoma cruzi*, the protozoan parasite responsible for Chagas disease, endocytosis is crucial for various aspects of its lifecycle. During the epimastigote stage, present in the midgut insect vector, the parasite can endocytose a wide range of macromolecules through the cytostome-cytopharynx complex. These macromolecules are then delivered to endosomal compartments and reservosomes, which are lysosome-like organelles. The endocytosis of macromolecules supplies the cell with nutrients for replication, and starvation act as a trigger for differentiation into infective metacyclic trypomastigotes. The clinically relevant amastigote stage, which develops intracellularly in the vertebrate host, reside in the host cell cytosol. They have access

to the host's cytosolic macromolecules and organelles. Although amastigotes possess a functional endocytic pathway, limited information is available regarding the macromolecules they can endocytose and their interactions with host organelles, from where they could scavenge molecules. Cholesterol performs most of the sterol found in intracellular amastigotes suggesting that they can scavenge cholesterol from the host cell. We investigate cholesterol traffic in infected host cells by using fluorescent cholesterol tracer, confocal and super-resolution microscopic analysis, and high-resolution electron microscopy. Our data suggests a participation of ER contact sites as platforms for cholesterol transfer to the parasite. Understanding the mechanisms of endocytosis in amastigotes is crucial for unraveling the intricate interplay between the parasite and the host cell, providing insights into the parasite's survival strategies and nutrient acquisition. The deciphering these mechanisms will lead to a valuable knowledge to develop targeted therapeutic interventions and enhance our understanding of Chagas disease. **Supported by::** Faperj; CNPq **Keywords:**amastigotes;endocytosis ;contact-sites.

RT.04-04 - **What are trypanosomatids presumably exclusive to insects doing in human hosts? A closer look at isolation of *Crithidia* parasites from visceral leishmaniasis patients in Sergipe, Brazil.**

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Reports about occurrence of monoxenous trypanosomatid infections in humans have been gradually increasing. By analyzing clinical isolates (CIs) from visceral leishmaniasis (VL) patients in Sergipe, Brazil, we have found that 51 out of 62 CIs are phylogenetically related to *Crithidia fasciculata*. In view of these surprising findings, we have endeavored to study these new parasite strains, integrating genomic and phenotypic approaches to characterize them. For this, we performed whole-genome sequencing (WGS) analysis of *Crithidia sp* LVH60A strain and assembled the genome in 38 chromosomes. Genome alignment between LVH60A and *C. fasciculata* revealed an average nucleotide identity (ANI) of 93%, which is low to be considered within of a same species. Also, we performed WGS analysis of another 47 *Crithidia* CIs from VL. Read mapping rate of the 47 CIs to *C. fasciculata* was ~72%, whereas to LVH60A genome was ~ 99%. Cell growth at 25°C of LVH60A was similar to *C. fasciculata* (doubling-time of 9.6 and 7.81 h, respectively), whereas *L. infantum* doubling time was 15.2 h. Cell growth at 35°C of LVH60A was similar to *L. infantum*, corroborating its thermotolerance feature. Morphological analyses of cultured parasites were performed using scanning and transmission electron microscopy. *In vitro* infections were performed using murine and human cell lines and parasite load analyzed at post infection periods. The average rate of infection for *Crithidia sp* was 27%, whereas for *L. infantum* and *C. fasciculata* were 40% and 5%, respectively. In addition, dual RNA-seq analyses of human macrophages infected with different *Leishmania* and *Crithidia* strains during time-course infection will help to identify transcriptomic changes in the host and the parasite, assessing the differences between each type of parasite infection. We hope that the study of this new parasite will provide insights in the evolution of parasitism in Trypanosomatidae, besides understanding this new infection in humans. **Supported by**:: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP): 2016/20258-0 **Keywords**:Human Visceral Leishmaniasis;Crithidia sp LVH60A;whole-genome sequencing.

RT.05 – Progress in Vaccine Development and Diagnostic tests for parasitic diseases

RT.05-01 - *Toxoplasma gondii* vaccines: The past, present, and future

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Toxoplasma gondii is a protozoan parasite with worldwide distribution, which may be found in animals from the arctic, rain forest, arid zones, and even in marine mammals. Toxoplasma gondii normally causes a subclinical infection in most animal species, however, a primary infection during pregnancy can cause fetal pathologies, as well as abortions in humans and some animal species. . Additionally, in spite of the highly effective antiretroviral therapy (HAART) there is an estimated incidence of toxoplasmic encephalitis (TE) occurring in 16% of human immunodeficiency virus infected patients. Ocular toxoplasmosis (OT) is another concern with *T. gondii* infection and this may occur following congenital and acquired transmission, and the risk of OT is highly variable depending on geographic region, ranging from 2% (Europe and North America) to 18% (southern Brazil). Moreover, there are reports that *T. gondii* may be associated with psychiatric disorders, and may affect human behavior, personality and other phenotypic traits.
The main infection sources for human beings are through consumption of either vegetables or water contaminated with sporulated T. gondii oocysts, and undercooked meat infected with tissue cysts. Therefore, based on the longevity of tissue cyst in pork, which can remain more than two years, and that pork is one of the most important sources of T. gondii infections for humans, developing a vaccine against *T. gondii i*n pigs would be very desirable, and focused on tissue cyst reducing. Another important factor in the epidemiology of toxoplasmosis is the definitive host. Cats assume an important role due to the close interaction with human beings; infected cats shed millions of oocysts in the feces that contaminate the environment. The risk of infection via sporulated oocysts in human populations has been well documented. These data demonstrate the need to control oocyst shedding by cats, however, few studies have been conducted with this aim. Treatments are available to reduce clinical signs but there are no drugs available that kill the parasite or cure the host from infection. There is just one commercial vaccine available (Toxovax®, MSD Animal Health) that is used in United Kingdom, New Zealand, France, and Ireland. This vaccine comprises live tachyzoites of the incomplete S48 strain that has lost the ability to differentiate into tissue cysts in animals. The vaccine is licensed only for use in sheep and goats to be administered prior to mating, however, there are some concerns about its safety as the vaccine can infect humans and as it is a live vaccine it does have a short shelf life. Others live vaccines (RH, and T263) have shown protection against toxoplasmosis, but these carry the risk of reverting to virulence. Data obtained from Medline databases (PubMed, NCBI) using the key words "*Toxoplasma gondii*"; and "vaccine";, focusing on the years 2019, 2020, 2021, 2022, and 2023 (until July 2023) revealed that approximately 366 articles addressing vaccines against *T. gondii* were published, 80 in 2019 , 83 in 2020 , 86 in 2021 , 74 in 2022, and 43 in 2023. Most of these studies used mice (50 %, 184/366) as an experimental model, two used sheep, two in pigs, and three in cats. An important factor in *T. gondii* is that the main route of infection of the host is oral, and oocysts are the main form of infection for herbivores, and tissue cysts are a route of infection for pigs and people, so the local immunity in the gut via lymphocytes (mainly intraepithelial lymphocytes and IgA) are of fundamental importance in host resistance to the parasite. The focus of this presentation is to present the past, present, and future of the epidemiology, biology, parasite diversity and host-parasite interactions of *T. gondii* and to review current vaccination strategies further to the recent papers and the prospects for future vaccines against this parasite. **Keywords:**Toxoplasmosis;livestock, cats, pigs;animals, humans.

RT.05-02 - **Non-clinical toxicity and immunogenicity evaluation of repeated administration of a *Plasmodium vivax* malaria vaccine in mice and rabbits**

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Malaria caused by Plasmodium vivax is a pressing public health problem in tropical and subtropical areas. However, little progress has been made toward developing a *P. vivax* vaccine, with only three candidates being tested in clinical studies. We previously reported that one chimeric recombinant protein (PvCSP-All epitopes) containing the conserved C-terminus of the *P. vivax* Circumsporozoite Protein (PvCSP), the three variant repeat domains, and a Toll-like receptor-3 agonist, Poly(I:C), as an adjuvant (polyinosinic-polycytidylic acid, a dsRNA analog mimicking viral RNA), elicits strong antibody-mediated immune responses in mice to each of the three allelic forms of PvCSP. In the present study, a non-clinical safety evaluation was performed to identify potential local and systemic toxic effects of the PvCSP-All epitopes combined with the Poly-ICLC (Poly I:C plus poly-L-lysine, Hiltonol®) or Poly-ICLC alone subcutaneously injected into C57BL/6 mice and New Zealand White Rabbits after a 21-day recovery period. Overall, all observations were considered non-adverse and were consistent with the expected inflammatory response and immune stimulation following vaccine administration. Vaccine-induced antibody responses and vaccine-specific antibodies were detected in developmental toxicity studies in mice and rabbits. Mice that received the vaccine formulation were protected after the challenge with *Plasmodium berghei* sporozoites expressing CSP repeats from *P. vivax* sporozoites (Pb/Pv-VK210). In conclusion, in these non-clinical models, repeated dose administrations of the PvCSP vaccine with a Poly-ICLC adjuvant were immunogenic, safe, and well tolerated. **Supported by::** FAPESP (2012/13032-5) and INCTV

RT.05-03 - **Exploring miRNAs as potential biomarkers using an experimental model of chronic Chagas disease cardiomyopathy**

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MicroRNAs are small non-coding RNA molecules that play a crucial role in post-transcriptional gene regulation. They have been implicated in various biological processes, including development, differentiation, and disease pathogenesis. Emerging evidence suggests that miRNAs are involved in the pathogenesis of Chagas disease (CD), being associated with key processes, such as immune response modulation, cardiac remodeling, and parasite persistence. During the chronic phase of CD, miRNAs have been implicated in the regulation of cardiac remodeling processes, including fibrosis, hypertrophy, and apoptosis. Altered miRNA expression profiles have been observed in the hearts of Chagas disease patients, suggesting their involvement in the progression of cardiac pathology.
We investigated the miRNA transcriptome profiling in the cardiac tissue of chronically *T. cruzi*-infected mice treated with a suboptimal dose of benznidazole (Bz), the immunomodulator pentoxifylline (PTX), or the combination of both (Bz+PTX), following the CCC onset. At 150 days post-infection, Bz, PTX, and Bz+PTX treatment regimens improved electrocardiographic alterations when compared with the vehicle-treated animals. miRNA Transcriptome profiling revealed considerable changes in the differential expression of miRNAs in the Bz and Bz+PTX treatment groups compared with the control group. We observed that *T. cruzi* infection induced the upregulation of miR-146b-5p expression in the heart tissue of chronically infected mice and in *in vitro* cultivation of rat cardiomyoblasts (H9C2 cells), which was reversed upon Bz and Bz+PTX treatment regimens. Understanding the role of miRNAs in Chagas disease could have important implications for the development of novel diagnostic and therapeutic strategies. By targeting specific miRNAs, it may be possible to modulate immune responses, prevent cardiac damage, and interfere with parasite persistence.
Supported by:: CNPq, FAPERJ, Fiocruz (INOVA**)** **Keywords**:Chagas disease cardiomyopathy;Micro RNAs;Biomarker.

RT.05-04 - **LeishID: LAMP-based molecular diagnostic tool for species-specific *Leishmania* detection**

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Point-of-care (PoC) molecular differential diagnostic of leishmaniasis is urgently need since it can be misdiagnosed for example as sporotrichosis. Additionally, recent reports reveal the presence of dermotropic-associated species causing visceral leishmaniasis (VL) and vice-versa. Thus, a test able to concomitantly identify the *Leishmania* species is important to better understand epidemiology and physiopathology. In this regard, we developed the LeishID, a LAMP-based molecular tool able to detect small quantities of parasite DNA and differentiate among *L. amazonensis*, *L. infantum* and *L. braziliensis*. The probes were designed based on a *Leishmania* pangenome approach, where we selected species-specific DNA sequences filtered on the accessory genome. The LAMP reaction can be detected by naked eye, since the colorimetric output is based on the buffer acidification, leading to a yellow color, during DNA amplification in the positive test. Depending on the target, positive reactions can be detected as soon as 15 min at 65 ºC. However, 40 min incubation, increases test sensitivity. The LAMP test was able to detect as low as 1 pg of extracted *Leishmania* DNA for all tested species. Species-specific sets of primers were able to detect the species they were designed for without cross-reactivity among them neither on mammalian DNA. Clinical validation using spleen biopsies of dogs with VL, samples derived from skin lesions of cutaneous leishmaniasis from human patients and Phlebotominae sandflies, revealed sensitivity varying from 93-97% and specificity of 97-10%. Thus, LeishID is a PoC compatible solution, rapid, specific, and sensitive to differentiate *Leishmania* species in clinically relevant concentrations.
Supported by:: Fundação de Amparo à Pesquisa do Estado de Minas Gerais - Fapemig (RED-00032- 22) e Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq - (312965/2020-6) **Keywords:**Leishmania;LAMP;pangenome.

RT.06 – Genomics

RT.06-01 - **Genomes of *Endotrypanum schaudinni* and *Zelonia costaricensis*: Expansion of multigene families in Leishmaniinae parasites that are close relatives of *Leishmania* spp.**

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The Trypanosomatidae family consists of obligate parasites of a range of organisms, including vertebrates, invertebrates, and plants. As such, genomic plasticity is an essential capability in order to adapt to new hosts and conditions. The Leishmaniinae subfamily is especially interesting in that it presents lineages that are either monoxenous or dixenous parasites, enabling comparative studies aiming towards a better understanding of the evolution of parasitism and lifestyle. Here, we focus on *Zelonia costaricensis* and *Endotrypanum schaudinni*, considered some of the nearest relatives of the genus *Leishmania*, an important parasite to humans. Parasite genomes evolve by employing gene repertories that can expand (gain genes) or contract (lose genes) depending on environmental pressures, which, in the case of the trypanosomatids, is their host (or hosts). We have used comparative genomics methods to identify ortholog groups (OGs) shared among 27 different trypanosomatid genome, then selecting OGs that were specific of our taxa of interest. The *E.*

schaudinni and *Z. costaricensis* genomes were assembled and display sizes of 29.9 Mb and 38.0 Mb, with 9,711 and 12,201 protein-coding genes predicted, respectively. Furthermore, *E. schaudinni*, a dixenous parasite, displayed a high number of multicopy genes, which include for example gp63 and gp46. Conversely, *Z. costaricensis* presents expansions of BT1 and amino acids transporter genes. We have also confirmed the evolutionary relationships of both species within the subfamily Leishmaniinae by using supermatrix (3,984 protein coding genes) and supertree methods. Overall, this study showed new expansions of multigene families into monoxenous and dixenous parasites of the Leishmaniinae subfamily. **Keywords:**genomics;Trypanosomatidae;genome evolution.

RT.06-02 - **VEuPathDB: Omics support for the global parasite, vector and fungal research communities**

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VEuPathDB (https://veupathdb.org), is a family of free, online bioinformatics resources supporting >500 species including protozoan parasites, fungi and oomycetes, arthropod vectors and selected host species. VEuPathDB resources facilitate the discovery of meaningful biological relationships from large volumes of data by integrating pre-analyzed Omics data with advanced search capabilities, data visualization and analysis tools. Specialized analytical or computational skills are not required since tools are offered in a friendly web-interface and supported by an email help desk, video tutorials, webinars, and social media. Available data types include genome sequence and population-level variation data; manually-curated and automatically generated annotation; epigenetic, transcriptomic and proteomic data; and pathway information. In addition, geospatially resolved vector surveillance data is available in the VectorBase tool called MapVEu. To take advantage of data from other species, a phylogenetic framework facilitates cross-species functional inference via orthology. In addition, a Galaxy interface provides a bioinformatics platform for privately analyzing your own large scale data and porting your results to VEuPathDB for comparisons with public data. VEuPathDB's comprehensive data mining resources offer valuable in silico hypothesis development and testing, so that end users can answer questions concerning expression levels and timing, domain presence, gene model integrity and genetic variation before venturing into the laboratory. VEuPathDB is one of two NIAID-supported Bioinformatics Resource Centers and receives additional support from the Wellcome Trust. Please email help@veupathdb.org with questions or suggestions.
Keywords: Bioinformatics;Databases;Genomics.

RT.06-03 - **Mosaic aneuploidy in Leishmania and its role in adaptation**

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Aneuploidy, an imbalance in the copy number of chromosomes in a cell, is generally considered harmful, but in some microorganisms, it can act as an adaptive mechanism against environmental stresses. Leishmania emerged as a new model for aneuploidy studies as it has a plastic genome characterized by rapid and dynamic modulation of chromosomes copy number, which leads to different karyotypes co-existing even among sister parasites in clonal populations (mosaic aneuploidy). In our lab we use (single cell) genomics and other omics approaches to study the role of (mosaic) aneuploidy in adaptation. By combining genomics, transcriptomics, proteomics and metabolomics of highly aneuploid strains of Leishmania donovani we established that aneuploidy has a strong impact on the transcript- and protein abundance levels of affected chromosomes, ultimately correlating with the degree of observed metabolic differences.

Moreover, an indirect effect of aneuploidy could be observed through changes in the levels of proteins related to protein folding pathways such as chaperones, chaperonins and heat shock proteins. In the second series of experiments, we applied a high throughput single-cell genome sequencing (SCGS) technology to investigate the extent of mosaic aneuploidy in two distinct clonal L. donovani populations, in standard in vitro culture. We revealed for the first time the complete karyotype of thousands of individual Leishmania parasites, identifying hundreds of karyotypes co-existing in each population. Finally, by combining single-cell genomics, lineage tracing with cellular barcodes and longitudinal genome characterization we investigated the dynamics of mosaic aneuploidy during adaptation to high drug pressure (Sb III and miltefosine) in vitro. We found that aneuploidy changes under Sb III pressure result from the polyclonal selection of pre-existing karyotypes, complemented by further and rapid de novo alterations in chromosome copy number along evolution. In the case of miltefosine, early parasite adaptation was associated with independent point mutations in a miltefosine transporter gene and aneuploidy changes only emerged later, upon exposure to increased concentration of the drug. Thus, polyclonality and genome plasticity are hallmarks of parasite adaptation, but the scenario of aneuploidy dynamics is dependent on the nature and strength of the environmental stress as well as on the existence of other pre-adaptive mechanisms.

RT.06-04 - **Updated Genome Assemblies and Metagenomics of Phlebotomus papatasi and Lutzomyia longipalpis**

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Sand flies serve as vectors for several established, emerging and re-emerging infectious agents. As important vectors of human disease, phlebotomine sand flies are of global significance to human health, transmitting protozoan, bacterial, and viral pathogens, the most devastating which is leishmaniasis. Here we present high-quality chromosome-level genome assemblies of both Phlebotomus papatasi and Lutzomyia longipalpis. Single individual males were sequenced by PacBio HiFi using ultralow input library preparation and additional material was used to generate HiC data for scaffolding. The L. longipalpis genome size was estimated to be 148 Mb and assigned to four chromosomes with 53x coverage and no unplaced scaffolds. The genome coverage for P. papatasi was 14.1x with an estimatedgenome size of 352 Mb with five chromosomes and 640 unplaced scaffolds (26 Mb). Additionally, microbiome species were annotated from DNA sequences of individual P. papatasi females from Afghanistan, Tunisia, and Egypt and L. longipalpis males from five different populations in Brazil (Jacobina, Marajo, Sobral 1S, Sobral 2s, Laphina. In these field-collected sand flies, individuals separated into distinct clusters reflecting their collection site. Interestingly, the sympatric L. longipalpis Sobral 1S and Sobral 2S populations were more similar to allopatric populations than each other. This characterization of microbial communities in wild sand fly populations will allow further exploration of how the microbiome can be manipulated to alter vector-parasite survival and transmission efficiency. **Keywords:**Sand flies;Genome;Micro biome.