## HP-01 - **Iron and heme status at the Leishmania-host interface: effect of iron deficiency anemia on the Leishmania (L.) amazonensis virulence.**

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Leishmaniases, a spectrum of diseases caused by *Leishmania*, affect millions of people worldwide. During infection, nutrient availability within the phagolysosomes has significant effects on parasite replication and virulence. This process requires the acquisition of essential nutrients such as iron and heme from the host since *Leishmania* lacks iron storage proteins or the capacity to synthesize heme. Importantly, free iron and heme are cytotoxic as they trigger the generation of free radicals in the presence of oxygen. *Leishmania*, therefore, must acquire heme and iron to survive in a hostile environment that restricts the availability of nutrients to the pathogen, a process called nutritional immunity. Identifying the several proteins that participate in the transport of iron and heme was crucial for understanding these metabolic pathways in *Leishmania.* Here, we sought to investigate the cross-regulation between the two membrane transporters, *Leishmania* Iron Transport 1 (LIT1) and *Leishmania* Heme Response 1 (LHR1), and the effect of host iron and heme availability on *L. amazonensis* infection. We generated mutants overexpressing GFP-tagged LIT1 and LHR1 in WT and LHR1 SKO lines. These mutants are characterized regarding intracellular heme levels, promastigote growth profile under normal and heme-depleted conditions, and *in vitro* infectivity. Promastigote growth profile in heme-depleted conditions revealed that LIT1 overexpression may compensate for the loss of one LHR1 allele. Besides, numerous unsuccessful attempts to generate LIT1 KO by CRISPR-Cas9 indicate that, despite previous publications, LIT1 may be essential. At last, preliminary results on the impact of iron deficiency anemia on *L. amazonensis* infection in mice showed that iron-deficient anemic mice developed smaller cutaneous lesions than healthy mice. Such findings could contribute to in-depth knowledge of iron and heme metabolic pathways and unveil the importance of nutritional immunity on leishmaniases. **Supported by:**FAPESP 2021/03355-0
**Keywords:**leishmaniases;host-pathogen interaction;nutritional immunity.

## HP-02 - **Functional characterization of the Asparagine Synthetases of *Trypanosoma cruzi***

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During its life cycle, *Trypanosoma cruzi* undergoes morphological and biochemical changes to adapt to considerably different environmental conditions. In this respect, amino acids and their derivatives are part of the toolbox used by the parasite to cope with such conditions and it has been reported their paramount roles in biological processes other than protein biosynthesis, serving as carbon and energy sources, and participating in host cell invasion, differentiation and resistance to different types of stress. Regarding the role of Asn, we have shown its involvement in several cell processes. In this work we explore the functional role of the enzyme that catalyzes the production of Asn in *T. cruzi*. For this, using CRISPR-Cas9 system, we produced partial and total knockout lineages for the gene encoding the Asn Synthetase (TriTrypDB TcCLB.503625.10) and then phenotypically characterized evaluating processes such as proliferation, host cell infection and trypomastigotes liberation, metacyclogenesis, viability, mitochondrial respiration, resistance to oxidative stress and to accumulation of ammonia ($NH_4^+$). Although no significant differences in the epimastigoytes proliferation, cell viability, mitochondrial respiration and resistance to oxidative stress were observed for the mutant lineages, there were differences in the metacyclogenesis, since we observed an increase of about 30% in the number of trypomastigotes in the partial knockout lineages incubated in TAU+3AAG medium and an increase of about 20% when these parasites were incubated in TAU+Asn when compared to the control Cas 9; we also observed a decrease in the resistance to ammonia accumulation in the partial knockout lineages(IC50<30mM), and interestingly, an increase in the resistance (IC50>30mM) in the total knockout lineages. We intend to perform further evaluations of these lineages, currently we are producing the Add-back lineages along with the biochemical characterization of the recombinant protein. **Supported by:**Fundação de Amparo à Pesquisa do Estado de SP, Processo: 2022/12315-5 **Keywords:**Trypanosoma cruzi;Asparagine Synthetase;ammonia.

### HP-03 - **First report of Leishmania RNA virus 2 (LRV2) in Leishmania infantum strains from canine and human visceral leishmaniasis in the Southeast of Brazil**

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Protistan parasites of the genus *Leishmania* (Kinetoplastea: Trypanosomatidae) are causative agents of leishmaniasis, one of the most important vector-borne diseases prevalent in almost 100 countries. This disease is known to present a broad range of clinical manifestations, a result of multiple interactions between the parasite and the host immune system. However, despite the substantial efforts to unravel the molecular mechanisms governing this interactive interface, they are still not fully understood. A presence of endosymbiotic *Leishmania* viruses has been suggested as an important piece in this puzzle. *Leishmania RNA virus 1* (LRV1) in South American *Leishmania* are commonly found in parasites belonging to the subgenus *Viannia*, while *Leishmania RNA virus 2* (LRV2) were thought to be restricted to the Old-World pathogens of the subgenus *Leishmania*. In this work, we searched for LRV2 in 71 strains of *Leishmania* (*L.*) *infantum*, the causative agent of visceral leishmaniasis (VL), from distinct hosts, clinical forms, and geographical origins. Seventy-one isolates were examined for LRV2. RNAs were isolated from 1 x $10^8$ cells using the Trizol[TM] (Invitrogen) method following the manufacturer's guidelines (Life Technologies/Thermo Fisher Scientific, Carlsbad, CA). One hundred ng of each RNA sample was treated with DNAse I prior to complementary DNA (cDNA) synthesis. Reverse transcription was performed using the Super Script III- First Strand Synthesis Kit, with random hexamer primers following the manufacturer's specifications and LRV1/2 specific primers. Two strains, one isolated from the canine (CUR268) and from human (HP-EMO) cases in the southeastern of Brazil were positive for LRV2. To the best of our knowledge, this is the first detection of LRV2 in New World. **Supported by:**CNPq - 302972/2019-6
**Keywords:**Leishmania infantum;LRV2;endosymbionts.

### HP-04 - **Phase I and II of NasoLeish®, a novel vaccine against canine visceral leishmaniasis caused by *Leishmania infantum.***

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Visceral leishmaniasis is a lethal neglected disease caused by *Leishmania infantum* and *L. donovani*. In Brazil, dogs are the main reservoir of *L. infantum*. We designed a novel vaccine formulation, NasoLeish®, for intranasal administration to newborn puppies consisting of *L. amazonensis* antigens associated with retinoic acid nanostructured with pegylated liposomes as an adjuvant. Our pre-clinical tests in mice demonstrated the efficacy of intranasal NasoLeish® in preventing parasite growth in the spleen, liver and bone marrow after infection. Also, a clinical trial with 23 *L. infantum*-free mongrel dogs, non-reactive by SNAP, ELISA and PCR, was concluded in 2022. Dogs received two intranasal doses of NasoLeish® at 15- day intervals, or 3 subcutaneous doses of the marketed vaccine Leish-Tec® as controls. NasoLeish® showed to be immunogenic, increasing the serum levels of anti-promastigote IgG, IgA and IgM and anti-amastigote IgG. Of note, all dogs tested negative for the DPP rapid test 30 days after the last dose, indicative of vaccine non-interference with current CVL diagnostic test. We also demonstrated that the vaccine leads to an increased production of IFN-γ and TGF-β while reduces the production of IL-10. Dogs were challenged with *L. infantum* 3 months after vaccination and tested for PCR 2 months after infection. We could see that NasoLeish® strongly reduce the parasite burden at spleen, liver and bone marrow. Overall, NasoLeish® is a promising candidate for the prevention of canine visceral leishmaniasis caused by *L. infantum*. **Supported by:**FAPERJ, GCRF **Keywords:**Canine visceral leishmaniasis;Intranasal vaccine;Mucosa.

## PV-01 - **Role of α-tubulin acetylation in the cell cycle of *Trypanosoma cruzi***

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The most abundant isoform in all microtubular structures of Trypanosomatids is acetylated α-tubulin. Acetylation on K40 of α-tubulin is conserved from lower eukaryotes to mammals and is associated with microtubule stability. It is also known that it occurs significantly on flagella, centrioles, cilia, basal body and the mitotic spindle. The primary acetyltransferase that delivers this modification was recently identified as Mec-17/ATAT, a Gcn5-related N-acetyltransferase. Despite evidence supporting a role for K40 acetylation in microtubule stability, its biological function *in vivo* remains unclear. To study α-tubulin K40 acetylation we employed different genetic manipulation strategies in *Trypanosoma cruzi*. We analyzed the phenotypes of the resulting parasites using expansion, confocal and electron microscopy. Firstly we have expressed *Tc*ATAT in epimastigotes using the inducible vector pTcINDEX-GW. *Tc*ATAT is located in the cytoskeleton and flagella, colocalizes with acetylated α-tubulin in these structures and over-expression causes increased levels of the acetylated isoform and a halt in the cell cycle progression of epimastigotes. Also, these parasites become more resistant to microtubule depolymerizing drugs. Then we used the same system to over-express mutant versions of α-tubulin K40, which generates reduced levels of acetylated α-tubulin, quantified by flow cytometry. Also, the cell cycle progression is altered and an aberrant morphology is observed in these parasites. Finally we obtained *Tc*ATAT knock-out epimastigotes by CRISPR/Cas9, these parasite have undetectable levels of acetylated α-tubulin and severe defects in there replication rates, their motility is impaired and present a detached flagella. These evidence supports the idea that α-tubulin acetylation is crucial for *T. cruzi* replication and cell cycle progression and that *Tc*ATAT is responsible for this posttranslational modification. **Supported by:**Agencia Nacional de Promoción Cientifica y Tecnológica, Argentina (PICT2019-0526, PICT2021-0157), CONICET (PIBAA 1242)
**Keywords:**Cytoskeleton;Acetylation;Tubulin.

## PV-02 - **Development of paratransgenic *Lutzomyia longipalpis* by engineered bacteria driving refractoriness to *Leishmania infantum chagasi* infection.**

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The parasite *Leishmania infantum chagasi* is the etiologic agent of American visceral leishmaniasis (AVL), a disease transmitted by the sandfly *Lutzomyia longipalpis.* The use of insecticides to control vector populations is one of the main strategies to limit the spread of AVL. However, this approach has limitations, including drug resistance and growing socioecological impact due to the need for more potent compounds. The current scenario of territorial expansion with increasing incidence and lethality of AVL highlights the need for new strategies to control the spread of this disease. Paratransgenesis is a approach in which symbiotic bacteria of insect vectors are engineered to drive antiparasitic effector molecules and reduce their vectorial capacity. To find candidate bacteria for genetic manipulation, we isolated culturable bacteria that were enriched in the gut of *L. longipalpis* after blood feeding. We are identifying these symbionts through 16S rRNA sequencing and working to establish a genetically engineered lineage. We determined that 16µM of the antimicrobial peptide (AMP) Melittin was toxic to *L. infantum chagasi* axenic cultures and in artificially infected *L. longipalpis* females, but had no deleterious effect on the vector or bacterial symbionts isolated from their gut. Now we are testing the combination of other AMPs with Melittin to observe the occurrence of a synergistic anti-leishmanial activity. We are genetically engineering *Escherichia coli* strains C600 and BL21 with a plasmid expression vector encoding the PhoA signal peptide to express and secrete the AMP Melittin constitutively. This plasmid stability and Melittin secretion are being assayed in *E. coli.* Our next steps include testing the effect of transgenic *E. coli* conditioned media on *L. infantum chagasi* cultures and during their development in *L. longipalpis.* In this way, we propose the development of a paratransgenic sandfly with reduced vetorial capacity to transmit the *L. infantum chagasi.***Supported by:**CAPES **Keywords:**Lutzomyia longipalpis;Leishmania infantum chagasi;Paratransgenesis.

## PV-03 - **The three-dimensional genome organization in *Trypanosoma cruzi***

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In *Trypanosoma cruzi*, gene expression is broadly determined by post-transcriptional mechanisms. However, a significant decrease in global transcription has been observed to distinct life forms, suggesting some layer of transcription regulation (Elias *et al.,* 2001). The genome of *T. cruzi* is compartmentalized: conserved genes form the core compartment, and the multigenic family of genes encoding surface proteins composes the disruptive compartment remarked by synteny break (Berná *et al.,* 2018). Previously, we found core and disruptive genes preferentially located at open and closed chromatin regions, respectively (Lima *et al.,* 2020). The present work aims to understand how different genomic features are organized into 3D genomic structures and to what extent the nuclear architecture influences gene expression. We evaluated the *T. cruzi* genome-wide chromosome conformation capture (Hi-C) public data (Tarleton, *et al.,* 2018) against the following set of genomic features: compartments, small RNAs, transcription start and end sites, pseudogenes and DNA repeats. We characterized core and disruptive rich Topological Associated Domains-like structures (TAD-like), in which disruptive rich TAD-like domains cover shorter genomic regions. We detected tDNAs, pseudogenes and transcription termination sites preferentially located at the boundary of TAD-like domains. Currently, we are investigating the enrichment of genomic features at TAD boundaries in between i. two open-open or closed-closed chromatin regions; or ii. in between two open-closed chromatin regions. This 3D genome organization suggests a range of chromatin folding patterns and opens a new question that we aim to address: "is gene expression impacted by the positioning of genomic features at the boundaries of TAD-like domains?" **Supported by:**FAPESP 2021/03219-0 **Keywords:**genome-wide chromosome conformation capture;3D genome organization;T; cruzi.

## PV-04 - **Iron Uptake Controls *Trypanosoma cruzi* Metabolic Shift and Cell Proliferation**

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(1) Background: Ionic transport in *Trypanosoma cruzi* is the object of intense studies. *T. cruzi* expresses a Fe-reductase (TcFR) and a Fe transporter (TcIT). We investigated the effect of Fe depletion and Fe supplementation on different structures and functions of *T. cruzi* epimastigotes in culture. (2) Methods: We investigated growth and metacyclogenesis, variations of intracellular Fe, endocytosis of transferrin, hemoglobin, and albumin by cell cytometry, structural changes of organelles by transmission electron microscopy, $O_2$ consumption by oximetry, mitochondrial membrane potential measuring JC-1 fluorescence at different wavelengths, intracellular ATP by bioluminescence, succinate-cytochrome c oxidoreductase following reduction of ferricytochrome c, production of $H_2O_2$ following oxidation of the Amplex® red probe, superoxide dismutase (SOD) activity following the reduction of nitroblue tetrazolium, expression of SOD, elements of the protein kinase A (PKA) signaling, TcFR and TcIT by quantitative PCR, PKA activity by luminescence, glyceraldehyde-3-phosphate dehydrogenase abundance and activity by Western blotting and $NAD^+$ reduction, and glucokinase activity recording $NADP^+$ reduction. (3) Results: Fe depletion increased oxidative stress, inhibited mitochondrial function and ATP formation, increased lipid accumulation in the reservosomes, and inhibited differentiation toward trypomastigotes, with the simultaneous metabolic shift from respiration to glycolysis. (4) Conclusion: The processes modulated for ionic Fe provide energy for the *T. cruzi* life cycle and the propagation of Chagas disease. **Supported by:**FAPERJ, E-26/203.901/2021 **Keywords:**ATP synthesis;mitochondrial function;parasite oxidative stress.

## PV-05 - **Mitochondrial inheritance in *Toxoplasma gondii* is dependent on actin and myosin A**

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*Toxoplasma gondii*'s single mitochondrion is highly dynamic and changes morphology when the parasite is exposed to different environments. This parasite divides by endodyogeny, where two daughter cells are formed inside of a mother cell. The organellar division is tightly regulated with the cell cycle, and the mitochondrion is the last organelle to be divided. Parasite's mitochondrial dynamic is dependent on a protein named **L**asso **M**aintenance **F**actor **1** (**LMF1**). LMF1 mediates mitochondrial dynamics by interacting with IMC10, a protein localized at the inner membrane complex (IMC). LMF1 and IMC10 form a unique tether between the mitochondrion and the IMC. As little is known about mitochondrial inheritance, we have used the LMF1/IMC10 interaction as an entry point to dissect the machinery behind this process. Using a yeast two-hybrid screen for putative LMF1 interactions, we identified Myosin A (MyoA) as a strong candidate. Although MyoA is known to be located at the parasite's IMC, ultrastructure expansion microscopy (U-ExM) shows that this protein accumulates around the mitochondrion in the late stages of division. Parasites lacking MyoA show defective mitochondrial morphology, and a delay in mitochondrion delivery to the buds during division, indicating that this protein is involved in organellar inheritance. Disruption of the parasite's actin network with Cytochalasin D also affects mitochondrion morphology. We have shown that parasite-extracted mitochondrion vesicles interact with actin filaments. Interestingly, mitochondrion vesicles extracted out of parasites lacking LMF1 pulled down less actin, showing that LMF1 might be important for mitochondrion and actin interaction. Accordingly, we are showing for the first time that actin and Myosin A are important for Toxoplasma mitochondrial inheritance. Current work includes characterizing other proteins involved in mitochondrial inheritance and how MyoA affects organellar speed during mitochondrion segregation. **Supported by:**NIH grants R01AI123457, R01AI149766, R01AI89808, and R21AI124067 to G.A. and in part, with support from the Indiana CTSI funded, in part by Grant Number UL1TR002529 from the NIH, NCATS, Clinical and Translational Sciences Award to R.O.O.S.
**Keywords:**Mitochondrion;Actin;Myosin A.

## PV-06 - **ACCESSING THE GENOMIC ELEMENTS AND STRUCTURAL VARIANTS BETWEEN *Crithidia sp* LVH60a AND *C. fasciculata* GENOMES**

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An increase in occurrence of monoxenic trypanosomatids, such as *Crithidia* and *Leptomona*s, has been observed in human cases of leishmaniasis. We have shown that some clinical isolates from VL (visceral leishmaniasis) cases in Sergipe, Brazil, do not belong to the *Leishmania* genus and are phylogenetically related to *Crithidia*, a monoxenous trypanosomatid considered non-pathogenic to humans. In order to characterize specific genomic elements and structural variations between *Crithidia sp* LVH60a-C1 strain and *C. fasciculata* reference, we performed whole-genome pairwise alignment analysis using MUMmer to calculate the average nucleotide identity (ANI) and Artemis to observe pairwise comparisons between the genomes of these two trypanosomatids. To validate the assembly chromosome features, we performed polymerase chain reactions utilizing LVH60a-C1 and TCC039E (*C. fasciculata*) strains. The alignment of LVH60a-C1 and *C. fasciculata* genomes exhibited an ANI ranging from 92.7% to 94.8%. We also observed chromosomal rearrangements of LVH60a-C1 when compared to *C. fasciculata* CfCl genome. We are validating arrangements in LVH60a-C1 genome by PCR regarding chromosomal positions of *C. fasciculata* as reference. So far, we validated two structural genomic arrangements in putative *Crithidia* LVH60a-C1 chromosomes: 1) Contig 25: 0.90 Mb syntenic to *C. fasciculata* chromosome 2 (0.53 Mb) fusioned with a 0.30 Mb fragment of *C. fasciculata* chromosome 29 (2.19 Mb); 2) Contig 12: partial duplication and inversion (0.04 Kb) of *C. fasciculata* chromosome 8. It is important to note that there is no established ANI threshold to classify trypanosomatids within the same species, however, previous studies have reported ANI values of around 94% between dermotropic and viscerotropic species of *Leishmania*. Our results may indicate a potential novel species within *Crithidia* genus, but more isolates need to be analyzed with focus on populational genomics to ascertain this evidence. **Supported by:**São Paulo Research Foundation (FAPESP) grant 2016/20258-0 and 2020/14011-8; CAPES; CNPq
**Keywords:**Crithidia sp;;comparative genome;chromosomal rearrangements.

## PV-07 - **Loss of RNase H1 in *Leishmania* leads to an altered DNA replication programme and increased genome variability.**

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Genome plasticity, manifested as gene and chromosome copy number variation, is an effective survival strategy used by *Leishmania* parasites and therefore stands as one of the stumbling blocks for developing effective anti-leishmanial therapies. However, our understanding about the mechanisms underlying genome malleability in this parasite remains limited. Here, we set out to investigate if and how R-loops contribute to genome variability in *Leishmania*. By using DRIP-seq, we show that a major locus for R-loop localization is at sites of coupled trans-splicing and polyadenylation, needed to generate mature mRNA. Globally, however, R-loop distribution within each *L. major* chromosome parallels the spatial and temporal compartments of the parasite's unconventional DNA replication programme. Strikingly, R-loop levels correlate with chromosome size, which in turn correlate with replication timing. In addition, R-loop levels are lower in subtelomeres, where replication can occur outside S-phase. DiCre-mediated inducible *KO* of *RNase H1* reveals a transient and dramatic growth perturbation, with resulting null mutants displaying pronounced changes in gene expression as revealed by RNA-seq. By performing MFA-seq analysis, we also observed dramatic changes in the DNA replication programme upon *RNAse H1* deletion. We also performed whole genome sequencing and observed increased levels of genome-wide, chromosome-size dependent instability, including aneuploidy, SNPs and InDels upon *RNAse H1 KO*. Finally, ChIP-seq analyses shows that *L. major* RNAse H1 is enriched at origins of DNA replication and, to a lesser extent, at transcription boundaries. We hypothesise our data reveal a crucial role for *Leishmania* RNase H1 in controlling the DNA replication programme by maintaining proper levels of R-loop and place the DNA-RNA hybrids as a pivotal player in *Leishmania* genome plasticity. **Supported by:**Wellcome, MRC, European Commission-Marie Skłodowska-Curie Actions **Keywords:**DNA Replication and Repair;R-loops and RNAse H1;Genome Plasticity.

## PV-08 - **Diversification of Heterotrophic Microeukaryotes: Insights from Arcellinida and Related Amoebozoan Taxa**

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Heterotrophic microbial eukaryotes play a pivotal role in marine and terrestrial ecosystems, contributing to carbon and nutrient cycles. These microorganisms, capable of phagocytosis, act as predators on bacterial communities and other microeukaryotes, occupying a significant position in complex food webs. The origin and diversification of these heterotrophic microeukaryotes remain unclear. Fossil evidence and molecular data suggest that the emergence of predatory microeukaryotes and the transition to a eukaryote-dominant marine environment occurred around 800 million years ago. Vase-shaped microfossils (VSMs) represent the oldest known evidence of heterotrophic microeukaryotes in marine environments and terrestrial habitats. In this study, we investigate the early divergence and diversification of Arcellinida and related amoebozoan taxa using a relaxed molecular clock approach. Our phylogenomic analysis reveals a well-resolved tree of amoebozoan testate amoebae, including a monophyletic Arcellinida with three suborders and five infraorders. Through calibration using fossils and rigorous clock models, we estimate the timing of diversification of Arcellinida during the early Neoproterozoic, shedding light on the expansion of life during this period. Our results suggest a well-established complexity in shallow marine ecosystems, involving both phototrophic and heterotrophic microeukaryotes during the neoproterozoic, followed by an invasion of freshwater systems and subsequent diversification of Arcellinida in the Phanerozoic. The findings indicate the need for some revision of the classification of amoebozoan testate amoebae, with taxa reassigned to different orders. Overall, this study provides valuable insights into the evolutionary history and ecological significance of heterotrophic microeukaryotes in Earth's ecosystems. **Supported by:**FAPESP 19/22815-2 **Keywords:**Arcellinida;Phylogenomic;Fossils.

## PV-09 - **Unveiling a novel Isoprenoid Salvage Pathway in *Plasmodium falciparum*: new perspectives in fosmidomycin as an antimalarial**

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Malaria, predominantly caused by the *Plasmodium falciparum* pathogen, remains an endemic problem in tropical and subtropical regions. The emergence of drug resistance is a major problem, highlighting the need for new antimalarial compounds. Fosmidomycin (FOS) inhibits the methylerythritol 4-phosphate (MEP) pathway, crucial for isoprene unit biosynthesis. Despite its efficacy, recurrent cases of recrudescence have led to investigations into the biological causes of FOS recovery. This study found isoprenoid alcohols such as farnesol (FOH), geranylgeraniol (GGOH), phytol (POH), and unsaponifiable lipid extracts from foods can restore FOS activity in *P. falciparum*. These substances are phosphorylated, lengthened, and incorporated into proteins. Proteomic and radiolabeling studies showed that prenylated proteins can bind to several isoprenoids if externally supplied. A gene (Pfpolk) encoding a prenol kinase was identified, which when expressed in yeast, exhibited farnesol and geranylgeraniol kinase activities. Conditional knockout parasites (ΔPfpolk) were created using CRISPR-Cas9 and DiCre strategies, to investigate the biological importance of the farnesol/geranylgeraniol salvage pathway. ΔPfpolk parasites were more susceptible to MEP inhibitors and incapable of using isoprenoid alcohols for protein prenylation. The study suggests that the farnesol and geranylgeraniol salvage pathway is an additional isoprenoid source for malaria parasites. Inhibition of this pathway could enhance the effectiveness of drugs targeting isoprenoid metabolism. A compound reducing the FOS-recovering effect of geranylgeraniol was identified, making it a potential candidate for co-use with FOS in trials. Collectively, these findings deepen our understanding of the action mechanisms of antimalarials targeting the apicoplast and shed light on a novel post-translational modification of proteins in *P. falciparum*, providing valuable insights for the development of more effective antimalarial drugs.
**Supported by:**FAPESP, 2018/02924-9 e 2018/02924-9 **Keywords:**Malaria;Isoprenoid;Drug.

## PV-10 - **Molecular Insights into the Evolutionary Origin and Mechanisms of Shell Formation in Amoebozoan Testate Amoebae (Arcellinida:Amoebozoa)**

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The emergence of evolutionary novelties is a central question in evolutionary biology. Some amoeboid organisms have an outer covering (shell), and this feature is an evolutionary novelty in the group of Arcellinida testate amoebae. While arcellinid's shell formation process has been well documented in the literature based on morphological data, we currently lack an understanding of the molecular apparatus underlying this process, impairing the inference of the evolutionary events involved in its origin. Here we report a hypothetical model of the molecular pathway for the shell formation process derived from morphological descriptions and supported by de novo sequencing, assembly, and annotation of single-cell transcriptomes of Arcella intermedia. Our model suggests that the origin of the shell formation process involved the co-option, with gene duplication events, of the molecular machinery that controls cell polarization and regulative exocytosis in eukaryotes. Further investigation based on immunofluorescence and other methods will enable us to test the model in situ and describe the shell formation process molecularly. Ultimately, we aim to gain insights into the origin and evolution of the shell formation process in Arcellinida. **Supported by:**FAPESP - 2019/22692-8 **Keywords:**Evolutionary Cell Biology;Omics;Differential gene expression.

## PV-11 - **Emerging-3'UTR ncRNAs of duplicated genes in *Leishmania major*: what is their relevance?**

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Noncoding RNAs (ncRNAs) are transcripts with low translational potential that play a role in many different human diseases. Here, two ncRNAs (c12 and OOD3) emerging from the 3'UTR of duplicated genes in *Leishmania major* were investigated by multi-colour single molecule fluorescence *in situ* hybridisation (smFISH) in order to prove their presence and characterise location, expression levels, stability and biogenesis. For both predicted ncRNAs, smFISH detected about similar ratio of the ncRNA signal as free and presented within the 3´UTR of the cognate mRNA. ncRNA_c12 was preferentially cytoplasmic (~80%) while a similar distribution between nucleus and cytoplasm was found for the ODD3. Both ncRNAs were less stable than the coding genes they were derived from and, when found as 3'UTR constituent, did not affect the stability of their cognate transcripts. The levels of free ncRNAs in the cell decreased by blocking the trans-splicing for 30min, which can also be correlated to their short stability. However, the level of c12 co-localised with the cognate gene was not affect by trans-splicing inhibition, even at 2h post sinefungin treatment, directly related to its stability. By treating the total RNA with different RNA modification enzymes, we confirmed the presence of CAP at 5' end of ODD3 transcript. However, c12 was identified as partially capped, maybe related to the intracellular location of this ncRNA. Lacking or overexpressor parasites for ODD3 lead to consistent changes in the transcriptome, indicating possibly its action as a trans-regulatory RNA. All the evidence aggregates relevant and new insights about the function of these 3'UTR emerging ncRNAs, considered as mRNA sub-product so far. Interestingly, despite similar genomic origin, ODD3 and c12 have different biogenesis and processing. In some way, the parasite has peculiar and still unearthed machinery(ies) responsible to distinguish similar ncRNAs and give function for some of them. **Supported by:**FAPESP 2020/00088-9 / 2021- **Keywords:**noncoding RNA;RNA-FISH;Leishmania.

## PV-12 - **Recombinant Expression of *Trypanosoma cruzi* Histones for Nucleosomes Assembly**

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Chagas Disease, caused by the parasite ***Trypanosoma cruzi***, continues to afflict millions of individuals worldwide, lacking effective vaccines and proper treatment options for its chronic phase. The infection and survival of the parasite within the host involves the regulation of gene expression at the post-transcriptional level. Emerging evidence suggests that epigenetic mechanisms play a pivotal role in the biology of these parasites, presenting a promising approach for targeted drug discovery. To unravel the complex workings of these mechanisms, it becomes imperative to explore the chromatin organization, the atomic structure of histones and, therefore, the nucleosome core particles (NCPs). This study aims to undertake a comprehensive investigation into the structure of histones, produced through recombinant expression techniques, as well as the NCPs themselves, using Cryogenic Electron Microscopy (Cryo-EM) techniques. Moreover, it seeks to investigate the kinetic and thermodynamic properties governing NCP assembly and its interaction with DNA. To achieve these objectives, both canonical histones (H2A, H2B, H3, and H4) and variant histones (H2A.Z, H2B.V, H3.V, and H4.V) are being expressed with the aid of single-gene as well as polycistronic constructs to overcome instabilities in the natural folding of proteins. The biophysical aspects of NCP formation and its interaction with specific regions of ***T. cruzi***'s genome are being evaluated using advanced fluorescence anisotropy techniques. Structural investigations will be conducted using Cryo-EM in order to attain a comprehensive understanding of the organization and function of NCPs in ***T. cruzi***. The preliminary results obtained thus far underscore the critical importance of further studies to acquire the necessary knowledge regarding the genetic regulation of ***T. cruzi***, thereby laying the foundation for the development of innovative and effective drugs. **Supported by:**Agência financiadora: CNPq; Nº do processo: 130990/2022-1 **Keywords:**Cryogenic Electron Microscopy;Nucleosome Core Particle;Trypanosoma cruzi.

## TB-01 - **RNA BINDING PROTEINS AS TRANS-REGULATORS IMPACTING SURVEILLANCE AND INFECTIVITY IN *LEISHMANIA.***

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*Leishmania* spp. protozoan Kinetoplastids present peculiar gene expression fundamentally dependent upon post-transcriptional control. This elevates the importance of RNA binding proteins for gene regulation in these parasites. Building upon the mRBPome we isolated previously (Pablos, Ferreira et al., MCP, 2019), 70 mRNA-bound RBPs were selected from the three main *L.mexicana* lifecycle stages. A trans-regulator knockout clone library was created through barcoded CRISPR and screened for essential roles in cellular differentiation and macrophage or mouse infections. Of the 70 RBPs screened, 40 are essential candidates to cell viability and 18 contribute to lifecycle progression to human-infective stages and/or parasite infectivity. Examination of individual knockout lines for amastigote-specific mRBPs showed normal promastigote growth dynamics, whereas macrophage infection was inhibited or ablated, suggesting essential roles of RBPs for amastigote viability and virulence. Nine mRBPs were Immunoprecipitated and submitted to transcriptomic analysis for to identify associated transcript targets that may represent novel virulence factors. **Supported by:**MRC, GCRF, NTD **Keywords:**LEISHMANIA;RNA Binding Protein;INFECTION.

## TB-02 - **DEVELOPMENT OF AMPHOTERICIN B MICROPARTICULATED IMPLANTS BY SPRAY-DRYING FOR LOCAL TREATMENT OF CUTANEOUS LEISHMANIASIS**

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Localized cutaneous leishmaniasis (LCL) is the most common form of the disease. Intralesional injections with Glucantime antimonial have reduced the systemic toxicity produced by parenteral injections, but they are painful, often require repetition, and are only applicable in medical posts. We have envisaged to develop novel drug formulations to allow a single local injection to be effective in LCL. Previously, we showed the promising use of biodegradable PLGA (poly(lactide-co-glycolide acid) microparticles (Mcps) loaded with amphotericin B (AmB) for sustained drug release. Here, we proposed to improve the process of AmB/PLGA Mcps production by using the spray-drying instead of the previous emulsification-solvent evaporation method. Spray-drying allows scale-up production and the use of less toxic solvents and surfactants. Using an appliance equipped with a three-fluid nozzle set, we produced Mcps containing AmB in a shell-core design. Fabrication process showed 60-70% yield; average particle size of 13.5 µm; and >70% drug incorporation rate. MEV images showed spherical and rough particle topology. *In vitro* drug release kinetics showed that AmB/PLGA Mcps promoted much slower release than free AmB. DSC and FTIR analyses showed that the spray-drying process did not change AmB and PLGA physical and -chemical characteristics. Murine macrophages exposed to AmB/PLGA Mcps did not show signs of toxicity. Mice injected s.c. with AmB/PLGA showed a transient local inflammation as monitored by MEST and histopathology. BALB/c mice infected in the ear pina with *Leishmania amazonensis*-GFP and given a single s.c. injection with AmB/PLGA showed significantly smaller lesion growth and parasite burden as compared with free AmB (Anforicin B®). This study shows that the green spray-drying method can produce industrially feasible PLGA/AmB Mcp implants for an effective treatment of LCL with a single injection with AmB, and possibly with other newly discovered antileishmanial drugs. **Supported by:**CAPES nº 88887.374118/2019-00 e VALE S.A. **Keywords:**Cutaneous leishmaniasis;Amphotericin b;Spray-drying.

## TB-03 - **A genome-wide yeast surface display screen to identify vaccine targets for Chagas disease**

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Chagas disease (ChD) affects roughly 8 million people and is caused by infection with the protozoan parasite *Trypanosoma cruzi.* The resulting pathology begins with an acute infection presenting mild flu-like symptoms and then progresses to the chronic stage, which can be lethal. There are no vaccines available against ChD, and the existing drugs – nifurtimox and benznidazole – are highly toxic with limited efficacy in the chronic stage. We developed a *T. cruzi* genome-wide library for yeast surface display (YSD) to identify immunogenic proteins for vaccine development. Nanopore sequencing of the library showed a 30-fold coverage of the parasite genome with all parasite genes included and library fragments ranging between 0.5 to 3 kb. Computational prediction of the *T. cruzi* library-expressed proteins in the YSD system based on nanopore sequencing showed over 248,946 unique polypeptides. We confirmed successful yeast surface protein expression by flow cytometry and Western blotting. To identify immunogenic polypeptides, we performed five rounds of Magnetic Activated Cell Sorting (MACS) against sera of chronic ChD or healthy patients. About 40% of the library was enriched with sera of chronic ChD patients, and nanopore sequencing of libraries identified a set of ~6,600 ChD-specific immunogenic polypeptides ($p<0.05$; fold change > 2) at nucleotide resolution. The dataset revealed immunogenic peptides of proteins that react specifically to ChD antibodies and those cross-reacting with sera of healthy individuals. The libraries are useful resources for screening drug targets, protein ligands, vaccine targets, and biomarkers. The identified immunogenic antigens are being used for mice mRNA and recombinant protein vaccination studies. **Supported by:**International Development Research Centre (IDRC, 109929-001 to I.C.); Canada Foundation for Innovation (CFI, JELF 258389 to I.C.) **Keywords:**Chagas disease;vaccines;genome-wide yeast surface display screen.

## TB-04 - **Whole genome analysis of paromomycin resistant lines and susceptible isolates of *Leishmania amazonensis* reveals changes in chromosomal somy and variable polymorphisms**

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*Leishmania amazonensis* is the second most prevalent species that causes tegumentary leishmaniasis (TL) in Brazil. The available treatment is limited to the use of few drugs, that are mostly toxic and induce several side effects, in addition to the high-cost treatment. Paromomycin (PM) is a low-cost aminoglycoside antibiotic currently used in the chemotherapy of visceral leishmaniasis in Southeast Asia. Due to the potential of PM against *L. amazonensis* demonstrated in *in vitro* and *in vivo* studies (Coser et al., 2020; Coser et al., 2021) and the limitations of the current treatment, our aim is to identify potential genes associated with PM resistance and susceptibility in this species, using PM-resistant lines selected *in vitro* and two clinical isolates highly susceptible to PM. We sequenced *de novo* the genome of *L. amazonensis* reference strain (M2269), whose sequence is not completely assembled. The whole genome sequence of this strain was obtained through assembling of reads obtained from Nanopore and Illumina platforms and used as reference for investigation of short-nucleotide variants (SNVs), including single and multiple nucleotide polymorphisms and insertions/deletions in coding sequences, and chromosomal ploidy of PM-resistant lines and isolates that were sequenced using Illumina platform. Bioinformatic analyses showed differences in chromosomal ploidy in PM-resistant lines selected through stepwise selection and PM-susceptible isolates compared to the parental M2269, while lines selected by *in vitro* mutagenesis did not show significant changes in ploidy. Preliminary results indicated an average of 800 SNVs between PM-resistant lines and 9,500 SNVs among isolates. This study will contribute for the identification of genes associated with resistance and susceptibility to PM that can be useful to understand the molecular basis of the mechanism of action and resistance in *Leishmania*. **Supported by:**Processos FAPESP 2016/21171-6 e 2019/22175-3; Processo CNPq 405235/2021-6 **Keywords:**Drug resistance;Paromomycin;Whole genome sequencing.

### TB-05 - **Paving the road for genomic surveillance of leishmaniasis: source tracing of *L. donovani* in recent outbreaks of visceral leishmaniasis in West Nepal**

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Molecular surveillance of parasitic diseases may provide information of highest relevance for control programs, such as (i) following the evolution of epidemics in time and space, (ii) characterization of new transmission cycles, (iii) outbreak studies and source identification and (iv) detection of new variants with new clinical features. Currently, no molecular surveillance exists for leishmaniasis, despite the existence of suitable technologies. We previously showed the feasibility and significant added value of direct parasite whole genome sequencing (SureSelect-sequencing, SuSL) in host or vector. We further optimized SuSL for 3 different parasite species and different types of clinical samples. Here, we demonstrate the proof-of-principle of SuSL for genome surveillance, in the context of a recent outbreak of visceral leishmaniasis in Western Nepal. Blood samples were collected in 2019 and stored on DNA/RNA Shield. Three samples with highest amounts of DNA, positive for *Leishmania* and from different districts were sequenced, with a high genome coverage. The 3 genomes were compared to our database of *L. donovani* genome sequences in the Indian sub-continent: this revealed that 'outbreak parasites' were distinct from the 'core' population observed between 2002 and 2011 in the lowlands of Nepal, India and Bangladesh. One sample branched close to ISC1, a small population of parasites found more frequently in Nepalese highlands and likely functionally different from the 'core' parasites. Two samples clustered together with a divergent genome previously reported only once. We looked for possible signatures of drug resistance and found several missense mutations in known drug transporters (AQPs and LdMT). Altogether, our results support the need for further genomic surveillance, in particular in the context of the current elimination program in the Indian subcontinent and demonstrate the applicability of SuSL to molecular surveillance of blood. **Supported by:**Belgian Development Cooperation ; Department of Economy, Science and Innovation (Flanders) ; EU-MSCA-RISE (Leishield) **Keywords:**Post-elimination surveillance;Indian sub continent;Genome capture.

### TB-06 - **Antileishmanial of Drugs Identified by High Content Screening against Intracellular Amastigotes**

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Leishmaniasis is caused by several species of the protozoan parasites of the genus *Leishmania*. This disease treatment remains difficult, since the available drugs have shown to be highly toxic, besides several effects has been shown. Thus, new therapeutics are urgently needed. High content screening represents an important tool in the drug discovery process, since it optimizes the chances of finding an active compound from a large number of candidates. So, the aim of this work was to evaluate the antileishmanial activity of some commercial drugs by high content screening targeting intracellular amastigotes. Bone marrow-derived macrophages (BMDMs) were plated in 96-well plates, and then infected with *L. amazonensis* expressing RFP. 3h post-infection, cells were treated with the drugs at 10 µM. Then, image acquisition was made 24h, 48h and 72h post-treatment. It was evaluated the antileishmanial activity of 2560 drugs in the initial screening. After that, were selected 80 compounds showing at least 50% of intracellular amastigotes inhibition when compared to controls (treatment with DMSO 1%). After two revalidation assays, 38 still demonstrated antileishmanial activity. Following, these compounds have determined their $EC_{50}$ and $CC_{50}$ values, at this point 26 compounds that demonstrated $EC_{50}$ < 10 and SI > 10 were selected. These 26 selected compounds were repurchased lyophilized and re-tested against intracellular amastigotes, in this step 18 compounds maintained the antileishmanial activity. After other selection steps based on drug approval by regulatory agencies and route of administration, we selected 9 compounds FDA-approved and orally administered to follow in our *in vivo* studies. By the end, was found a drug called as C00 which reduced the ear thickness and parasite burden in ear after 15 days orally administered to mice infected with *L. amazonensis.* **Supported by:**CNPq - 152584/2022-6 **Keywords:**Antileishmanial drug;High content screening;Cutaneous leishmaniasis.

TB-07 - **Search for new Drugs and Potential Molecular Targets Associated with Benznidazole Resistance Phenotype in *Trypanosoma cruzi***

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Chagas disease (CD) is a public health problem in Latin America, caused by *Trypanosoma cruzi*. Nifurtimox and Benznidazole (BZ), the only two available interventions, have low cure rates during the chronic stage of the disease and present several toxic side effects. Recently, we performed a comparative transcriptomic analysis of wild-type and BZ-resistant *T. cruzi* lines, and the results revealed a robust set of genes from different metabolic pathways associated with the BZ-resistant phenotype. In this study, we are identifying new molecular targets and drugs against CD using computational methodologies and drug repositioning strategies looking for those that interact with differentially expressed proteins identified by transcriptomic analysis. The transcriptomic profile of *T. cruzi* revealed a list of 1,819 differentially expressed transcripts. Based on gene expression level and genomic context, we selected one gene for detailed investigation. This gene is unique to the Dm28c strain (TcI) and exhibits similarity to six targets in the DrugBank database. Using CRISPR/Cas9 system, we have successfully deleted one allele of this gene from Dm28c strain, and the deletion of second allele is in process. Among the identified molecular targets, we found 49 drug candidates for replacement, with 25 approved for medical use. The protein-protein interaction network consists of 7,685 proteins and 3,685,439 interactions. We have added node attributes such as resistance data and gene ontologies, and we are developing codes for simulations with network properties. The study of protein-protein interactions enables the identification of new molecular targets relevant to the resistance phenotype, even in the absence of increased expression. Therefore, the integration of *in silico* and *in vitro* approaches in the search for molecular targets and drugs can significantly contribute to the development of new treatments against CD. **Supported by:**Programa INOVA FIOCRUZ – Fundação Oswaldo Cruz (VPPCB-07-FIO-18-2-94); Convênio Fiocruz-Institut Pasteur-USP (no grant number); Fundação de amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG – APQ 02816-21 and RED-00104-22); Convênio UGA/FAPEMIG (APQ-04382 (D)); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 304158/2019-4) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – BRA (CAPES) – Finance code 001. **Keywords:**Drug resistance;Trypanosoma cruzi;Chemotherapy.