HP - 009 - Development and analysis of the trypanocidal potential of an Itraconazole nanoformulation

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Introduction: Current treatments for Chagas disease present important limitations, especially regarding efficacy during the chronic phase. Although the anti-T. cruzi activity of itraconazole has been previously demonstrated, its low water solubility limits systemic bioavailability. In this context, nanotechnology-based strategies have been explored to improve the solubility and delivery of lipophilic drugs. Lipid nanoformulations (LNs) stand out as promising drug delivery systems due to their biocompatibility and ability to encapsulate hydrophobic compounds.
Objective: To develop and characterize a nanoformulation with itraconazole and evaluate its potential trypanocidal activity in vitro. **Methods:** The LN was obtained by spontaneous emulsification (55 °C), followed by sonication (1 cycle of 15 min and 2 cycles of 5 min in an ice bath), using oleic acid and polyethylene glycol 400 to solubilize ITZ, in addition to monoolein and poloxamer. In triplicate, the LNs were characterized regarding mean hydrodynamic diameter (HD), polydispersity index (PDI), zeta potential (ZP), and stability over 90 days. Encapsulation efficiency (EE) and ITZ loading were determined by HPLC-UV (n = 3). Cytotoxicity was evaluated in vitro in L929 fibroblasts (50; 25; 12,5; 6,25; 3,125; 1,56 e 0,78 µg/mL) and trypanocidal activity was evaluated in L929 cells infected with T. cruzi Y strain parasites at the same concentrations (adapted from Romanha et al., 2010). **Results:** The LNs presented nanometric size, with an HD of 191.4 ± 1.47 nm, PDI of 0.202 ± 0.021, and ZP of −33.66 ± 1.01 mV, indicating good colloidal stability maintained over 90 days. EE was close to 100%, demonstrating high drug incorporation into the lipid matrix, although with low loading capacity (134.4 ± 1.5 µg/mL). A 100% inhibition of parasitic activity was observed at all tested concentrations, with an IC_{50} value lower than 1.17 µg/mL. However, none of the analyzed treatments showed a selectivity index higher than that of BNZ.
Conclusion: ITZ-loaded LNs showed promising physicochemical characteristics, with potential to optimize the systemic delivery of the hydrophobic drug, in addition to demonstrating trypanocidal activity. However, the low ITZ loading and the high cytotoxicity observed indicate the need for further optimization of the lipid nanoformulation to improve the safety and performance of the system for Chagas disease treatment. **Supported by:** CNPq, FAPEMIG, CAPES, UNIVERSIDADE FEDERAL DE OURO PRETO **Keywords:** Chagas diseases;Itraconazole;Lipid Nanoformulations.

HP - 010 - Humoral immune response during *Trypanosoma rangeli* infection in mice

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Although *Trypanosoma rangeli* is traditionally considered non-pathogenic to vertebrate hosts, recent evidence suggests that the parasite may establish prolonged infections in mammals. However, the host immune response associated with parasite persistence remains poorly characterized. Understanding the kinetics of humoral responses during infection may provide important insights into parasite-host interactions and mechanisms associated with long-term persistence. The present study investigates the humoral immune response during experimental *T. rangeli* infection in mice. Previous studies from our group demonstrated that *T. rangeli* establishes prolonged infection in Swiss Webster mice, with peak parasitemia at 5 days post-infection (dpi), detectable circulating parasites up to 90 dpi, and parasite DNA in spleen and mesenteric lymph nodes at 132 dpi. To characterize the humoral immune response associated with this persistent infection pattern, sera collected at 5, 10, 15, 30, 60, 90, and 120 dpi are being analyzed by flow cytometry to evaluate IgM, IgG, and IgG2a responses against *T. rangeli* epimastigotes. Longitudinal analysis of these isotypes enables characterization of adaptive immune response kinetics and investigation of its relationship with the observed parasite persistence. Evaluation of IgG2a levels may also provide insights into potential Th1-biased immune responses during infection. Preliminary findings suggest the development of a robust humoral response throughout infection, while cross-reactivity assays indicate low to moderate antigenic overlap with *T. cruzi*. Together, this study contributes to understanding the immunological mechanisms involved in the interaction between *T. rangeli* and the mammalian host, providing insights into how the parasite persists in lymphoid tissues despite the immune response.
Supported by: CAPES Keywords**:** Trypanosoma rangeli;Humoral immune response;Flow cytometry.

HP - 011 - CRISPR/Cas9-mediated genetic validation identifies Glycogen Synthase Kinase 3 (GSK-3) as an essential kinase controlling growth, stress adaptation, and virulence in Leishmania infantum

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Leishmania infantum depends on tightly regulated signaling pathways to maintain parasite growth, adaptation to stress, and infectivity. Glycogen synthase kinase 3 (GSK-3) is a conserved serine/threonine kinase implicated in multiple cellular processes, but its role in *Leishmania* remains poorly understood. This study investigated the function of the short isoform of GSK-3 (GSK-3s) in *L. infantum* using a CRISPR/Cas9-based genetic approach. Attempts to generate chromosomal GSK-3s null mutants were unsuccessful, suggesting that GSK-3s is essential for parasite survival. Gene disruption was achieved only after episomal complementation with *L. braziliensis* GSK-3s, generating a GSK-3s-deficient knockdown line (*LiΔchrGSK3::LbGSK3*). Quantitative PCR revealed a 3.8-fold reduction in GSK-3s transcript levels in this *L. infantum* line compared with control parasites. Reduced GSK-3s expression caused severe impairment of parasite proliferation, morphological alterations, and cell-cycle dysregulation. Knockdown parasites also exhibited increased resistance to trivalent antimony (5.3-fold), miltefosine (1.77-fold), and the GSK-3 inhibitor 6-bromo-5-methylindirubin-3′-oxime (6-BIO; 111-fold), supporting the specificity of this inhibitor toward parasite GSK-3s. In addition, GSK-3s-deficient parasites displayed enhanced tolerance to hydrogen peroxide-induced oxidative stress, accompanied by altered expression of antioxidant defense genes. Functional analyses demonstrated that reduced GSK-3s expression impaired parasite infectivity, resulting in lower early infection rates in THP-1-derived macrophages and significantly reduced splenic parasite burdens in BALB/c and IFN-$\gamma^{-/-}$ C57BL/6 mice. Collectively, these findings demonstrate that GSK-3s plays a central role in parasite proliferation, stress adaptation, drug response, and virulence. This study provides genetic evidence that GSK-3s is essential for *L. infantum* viability and highlights this kinase as a promising target for the development of new antileishmanial therapies. **Supported by:** Fundação de Amparo à Pesquisa do Estado de Minas Gerais, Fundação Oswaldo Cruz (Chamada de Redes Colaborativas de Pesquisa do Instituto René Rachou-Fiocruz Minas; Rede FarVac; Programa Inova Fiocruz – Edital Geração de Conhecimento, Conselho Nacional de Desenvolvimento Científico e Tecnológico and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior -Brasil (CAPES) and PIPD/CAPES. **Keywords:** Leishmania infantum; Glycogen synthase kinase 3 (GSK-3); Target validation.

HP - 012 - Standardization of fluorescence-based *in vitro* and *in vivo* drug screening using *tdTomato*-expressing *Leishmania amazonensis*

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Current chemotherapy for leishmaniasis is limited by toxicity, variable efficacy, and drug resistance, highlighting the need for reliable platforms for antileishmanial drug discovery. Here, we standardized fluorescence-based *in vitro* and *in vivo* assays using *Leishmania amazonensis* constitutively expressing *tdTomato* to enable rapid, sensitive, and noninvasive evaluation of antileishmanial compounds. Parasite susceptibility to trivalent antimony (Sb^{III}), amphotericin B, and miltefosine was assessed *in vitro*, while the *in vivo* model was validated in BALB/c mice treated with Glucantime®. Parasite burden was monitored by IVIS® imaging and compared with limiting dilution assays. No significant differences were observed between wild-type and *tdTomato*-expressing parasites regarding infectivity or drug susceptibility, indicating that *tdTomato* expression does not alter parasite biology. Fluorescence decreased significantly after treatment and correlated with parasite burden. The validated intracellular assay was then applied to screen 11 cinnamic acid-derived dipeptide mimetics and 60 crude plant and fungal extracts. Using a ≥ 50% inhibition threshold, 4 of the 11 (36.4 %) dipeptide mimetics and 12 of the 60 (20.0 %) extracts were classified as active. Among the extracts, *Clusia mexiae* showed the highest potency (EC_{50} = 13.1 µg/mL; SI = 16), followed by *Plinia nana* (SI = 15), whereas *Joannesia princeps* exhibited the highest inhibition (79.0%) but low selectivity (SI = 2). Among the synthetic compounds, 1b displayed the most favorable profile (61.2% inhibition, EC_{50} = 13.3 µg/mL, SI = 19), while 1k was the most potent (EC_{50} = 10.5 µg/mL; SI = 7). These findings demonstrate that *tdTomato*-expressing *L. amazonensis* is a robust platform for rapid *in vitro* and *in vivo* antileishmanial drug screening and identified *Clusia mexiae*, *Plinia nana*, and compound 1b as promising candidates for further development. **Supported by:** Fundação de Amparo à Pesquisa do Estado de Minas Gerais, Fundação Oswaldo Cruz (Chamada de Redes Colaborativas de Pesquisa do Instituto René Rachou-Fiocruz Minas; Rede FarVac; Programa Inova Fiocruz – Edital Geração de Conhecimento, Conselho Nacional de Desenvolvimento Científico e Tecnológico and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior -Brasil (CAPES) **Keywords:** Leishmania amazonensis;tdTomato;Drug screening.

HP - 013 - Establishing an experimental platform for genetic exchange in Leishmania (Viannia) using natural and permissive sand fly vector

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Natural hybrids involving *Leishmania braziliensis* demonstrate that interspecific genetic exchange occurs in nature, but whether hybridization can be reproduced under laboratory conditions remains untested. Although membrane-fusion compatibility has been shown in vitro and *L. braziliensis* infects *Lutzomyia longipalpis* under experimental conditions, no attempt has been made to recover hybrid progeny from experimental crosses in vivo. Because *L. braziliensis* and *L. infantum* co-circulate in overlapping endemic areas where they co-infect the same host, testing their reproductive compatibility is epidemiologically relevant. We hypothesize that intra- and interspecific hybridization is feasible and that reproductive compatibility is shaped by parasite biology, vector environment, or both. We are developing an experimental system using a dual-fluorescent, dual-drug selection strategy: each isolate expresses eGFP-Neo or mCherry-Sat, ensuring hybrids are double-fluorescent and double-resistant. Reporter lines representing both *L. infantum* and *L. braziliensis* have been generated to enable recovery of hybrid progeny. Membrane-fusion compatibility will first be screened in vitro by co-incubating gamma-irradiated promastigotes, selecting double-resistant parasites, and confirming double fluorescence by flow cytometry. Fusion-compatible pairs will be tested in vivo by performing intra- and interspecific crosses in *Lutzomyia longipalpis* and *Nyssomyia whitmani*, with co-colonization experiments assessing spatial distribution. Hybrid progeny will be recovered by double-drug selection and confirmed by dual fluorescence. A laboratory colony of *Lutzomyia longipalpis* has been successfully established at the Institute of Biomedical Sciences, University of São Paulo, and efforts are underway to establish a *Nyssomyia whitmani* colony. This work will establish an experimental platform to investigate genetic exchange in *Leishmania* (*Viannia*), testing whether laboratory hybridization is achievable and whether vector species influence reproductive compatibility. **Supported by:** FAPESP 2026/05579-7 Keywords**:** Genetic exchange;Sand fly;Leishmania (Viannia).

HP - 014 - Metabolite secretion patterns in Trypanosoma cruzi CL Brener epimastigotes revealed by specific carbon source exometabolomic profiling

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Trypanosoma cruzi, the causative agent of Chagas disease, is a metabolically versatile parasite capable of exploiting a wide repertoire of carbon sources across its complex life cycle. The epimastigote stage, which proliferates in the insect vector digestive tract, encounters environments of variable and often low nutrient availability. To systematically characterize the metabolic output of *T. cruzi* epimastigotes of the CL Brener strain under defined nutritional conditions, we performed a comprehensive ^{1}H-NMR-based exometabolomic analysis exposing parasites to 18 individual carbon sources, detecting 34 extracellular metabolites. Acetate emerged as the predominant secreted end product across the majority of carbon sources. Branched-chain amino acids (BCAAs; leucine, isoleucine, and valine) yielded their respective α-keto acid derivatives and downstream short-chain fatty acid products. Threonine catabolism generated the largest glycine excretion observed, consistent with L-threonine 3-dehydrogenase (TDH) activity. Cysteine metabolism produced large quantities of alanine and cystine, while methionine generated its α-keto acid 4-methylthio-2-oxobutanoate (KMTB). Histidine catabolism yielded urocanate and succinate, consistent with the operation of the four-step histidine → glutamate pathway. Glutamine and asparagine catabolism converged on alanine and succinate secretion. On the other hand, aspartate and glutamate produced a poor exometabolome, consistent with lower uptake rates respect to their amide analogues, explaining why amide forms generate rich metabolic profiles despite requiring an additional hydrolytic step before TCA entry. This exometabolomic map establishes a reference atlas of the *T. cruzi* CL Brener metabolic secretome and provides essential baseline data for genetic and pharmacological studies targeting this parasite's metabolic network. **Supported by:** FAPESP: grant to AMS N° 2021/12938-0, grant to MSB N° 2022/14959-7 Keywords**:** Trypanosoma cruzi;^{1}H-NMR exometabolomics;amino acid metabolism.

HP - 015 - Phenotypic characterization of attenuated Trypanosoma cruzi mutants deficient of active Trans-sialidases genes

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Trans-sialidases (*TS*) represents the largest multigenic family in the *Trypanosoma cruzi* genome with over 1400 gene copies, in which around 12 sequences encode enzymatically active TS (aTS), which are responsible for transferring sialic acid from host glycoconjugates to mucins present on the parasite surface, working as a mechanism of camouflage from the host immune response. Using CRISPR/Cas9 technology (premature stop codon insertion), we generated, aTS knockout parasites (aTS-KØ) that display undetectable levels of TS activity. aTS-KØ are unable to establish infection even in the highly susceptible IFN-ɤ KØ mice. Moreover, aTS mutants are highly attenuated and confer protection against virulent challenge. Although aTS-KØ parasites infect different cell types, they show impaired amastigote-to-trypomastigote differentiation and parasite release. To further investigate the role of aTS in infection aTS re-expressor lines (aTS-KØ AB) were generated by restoring the disrupted reading frame using CRISPR/Cas9. aTS-KØ AB localize aTS on the parasites surface and can partially rescue aTS activity, SA transference and in vitro phenotype with restoration TCT release from infected cells. In addition to their highly attenuated phenotype, aTS-KØ parasites exhibited a marked loss of tissue tropism in mice, particularly in the intestine, as assessed by bioluminescence-based ex vivo assays, which is rescued in aTS-KØ AB-infected mice. Histopathological analyses showed that animals infected with WT and aTS-KØ AB parasites exhibited marked inflammatory infiltrates, whereas tissues from aTS-KØ-infected animals displayed preserved architecture with no evident tissue damage, comparable to non-infected controls. These findings were confirmed by qPCR analyses, which showed the absence of parasite DNA in the tissues of aTS-KØ-infected animals (heart and intestine). Altogether, these data identify aTS as key virulence factors in *T. cruzi*, whose depletion yields a highly attenuated strain with vaccine potential.
Supported by: CNPq, FAPEMIG, INCTV, Capes **Keywords:** Active trans-sialidases (aTS);parasite tissue tropism;attenuated strain.

HP - 016 - Comparative Analysis of *Trypanosoma cruzi* and *Trypanosoma cruzi marinkellei* proteins following exposure to Cobalt-60 ionizing radiation

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The major challenges in combating Chagas disease are centered on diagnosis and treatment, underscoring the urgent need to develop a vaccine for disease prevention. Ionizing radiation has emerged as a promising technology for optimizing antigenic molecules. Here, we evaluated the effects of gamma radiation on the protein profiles of *Trypanosoma cruzi* and *Trypanosoma cruzi marinkellei*. SDS-PAGE analysis revealed that gamma radiation induced minimal protein alterations, even at the highest radiation doses evaluated. Regarding antigenicity, ELISA demonstrated robust antibody reactivity of human anti-*T. cruzi* IgG toward the irradiated proteins from both parasite species. Similarly, Western Blotting confirmed evident antigenic recognition by human anti-*T. cruzi* IgG against both native and irradiated parasitic proteins. Immunization of mice with native *Trypanosoma cruzi marinkellei* proteins induced the production of high-avidity antibodies cross-reactive with both *T. cruzi* and *T. cruzi marinkellei* antigens. Furthermore, Western Blotting showed strong antibody reactivity of sera from immunized animals against *T. cruzi* antigens. Altogether, our findings suggest that *Trypanosoma cruzi marinkellei* proteins are promising candidate molecules and that Cobalt-60 gamma radiation represents a viable strategy for vaccine development. **Supported by:** CAPES 88887.316945/2026-00 **Keywords:** Trypanosoma cruzi marinkellei;Trypanosoma cruzi;Ionizing Radiation.

HP - 017 - A flagellum-dependent cell-to-cell transmigration mechanism promotes *Trypanosoma cruzi* dissemination

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The ability of *T. cruzi* to persist at low levels during frequently asymptomatic, chronic infection remains a major barrier to effective disease control. Experimental mouse infection studies show that, during the acute stage, *T. cruzi* spreads rapidly throughout the host and disseminates to numerous tissues and organs. As infection progresses to the chronic stage, parasite foci become highly spatiotemporally dynamic, emerging and disappearing within hours. The mechanisms that enable *T. cruzi* to disseminate though host tissues and establish these transient foci remain poorly understood, representing a major gap in our understanding of parasite persistence and pathogenesis.
Combining molecular biology and live-imaging approaches to investigate host-parasite interactions, we identified a mechanism that promotes cell-to-cell parasite transmigration. This mechanism enables trypomastigotes to migrate rapidly, flagellum-end first, between host cells while acquiring coatings of host-derived plasma membrane. Through the generation of motility-deficient mutants, and the use of murine infection models, we demonstrate that parasite motility is required for normal dissemination *in vivo.* Together, these findings identify a previously unrecognized mechanism of parasite dissemination, and suggest that motility-driven cell-to-cell transmigration contributes to tissue colonisation, immune-evasion, and persistence during *T. cruzi* infection. **Supported by:** DNDi **Keywords:** Immune evasion;transmigration;motility.

HP - 018 - Early Intestinal Colonization Dynamics of *Trypanosoma cruzi* and *Trypanosoma rangeli* in Single and Coinfections of *Rhodnius prolixus*

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Trypanosoma cruzi and *Trypanosoma rangeli* are trypanosomatids that coexist in sympatry and naturally infect kissing bugs and mammals throughout the Americas. While *T. cruzi* remains restricted to the intestinal tract, *T. rangeli* crosses the gut epithelium, colonizes the hemolymph and salivary glands, and is transmitted during blood feeding. Their effects on the vector differ: *T. cruzi* generally causes mild alterations, whereas *T. rangeli* induces mortality, delayed development, and physiological and behavioral changes. The effects of natural coinfections on vector biology remain poorly understood. This study aimed to investigate the interactions between *Rhodnius prolixus*, *T. cruzi*, and *T. rangeli* during single and coinfections, focusing on parasite interactions within the insect gut. Parasite growth and temporal distribution were evaluated in different tissues of the vector. *R. prolixus* nymphs were infected by artificial feeding with 1×10^6 parasites/mL. In coinfections, 5×10^5 parasites/mL of each species were used, totaling 1×10^6 parasites/mL. At different time post-infection points, intestinal compartments were dissected, and parasite loads were estimated by fresh-mount microscopy using the Brener method. Exploratory data revealed distinct patterns of early intestinal colonization. *T. rangeli* showed higher parasite loads in the anterior midgut throughout the experimental period in both single and coinfections, whereas *T. cruzi* remained at low parasite loads in all intestinal compartments. In the posterior midgut and rectum, both parasites were detected less frequently and at lower parasite loads, particularly at later time points. These preliminary findings provide an initial overview of parasite colonization dynamics. Future qPCR Multiplex analyses will enable a more comprehensive assessment of the temporal distribution of *T. cruzi* and *T. rangeli* in different tissues of *R. prolixus*. **Supported by:** FAPEMIG **Keywords:** mixed infections;parasite-vector interactions;gut colonization.

HP - 019 - Transcriptional regulation of lncRNAs in murine macrophages correlates HIF-1α with coding and noncoding RNAs in response to *Leishmania* infection or LPS stimulation

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Leishmania infection induces broad transcriptional reprogramming in macrophages, integrating inflammatory, metabolic, and stress-response pathways. To investigate these layers of regulation, we analyzed RNA-seq data from WT and Hif1a-KO C57BL/6 macrophages infected with *L. donovani* for 6 h, as well as macrophages infected with *L. infantum* for 4 and 24 h. Our data reveal a dual transcriptional program in which HIF-1α regulates canonical immune pathways and specific lncRNAs. Notably, *Gm15283* was upregulated upon infection and LPS stimulation, while *Hif1a* knockout altered its expression. In silico analyses showed correlations between lncRNA expression and neighboring protein-coding genes, including *Il1a* and *Hilpda* lncRNA, suggesting dependence on HIF-1α-driven inflammatory signaling. Functional comparisons of lncRNAs and adjacent coding genes pointed to innate immunity, cytokine production, glycolysis, and tissue remodeling in both LPS and *Leishmania*-infected macrophages. Together, these findings support a model in which HIF-1α modulates both coding and noncoding transcriptional responses during *Leishmania* infection, revealing novel lncRNA-associated regulatory axes with potential roles in immune control and parasite restriction. **Supported by:** Fapesp - Processo 2022/08700-0; 2024/06233-1 **Keywords:** leishmaniasis;macrophages;lncRNAs.

HP - 020 - In Vitro and In Vivo Antileishmanial Activity of 3-Phenyl-Lawsone Loaded into PLGA Nanoparticles Against Leishmania braziliensis.

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American tegumentary leishmaniasis, caused primarily by *Leishmania braziliensis* in Brazil, is a neglected tropical disease. Current treatments face significant limitations, including toxicity, prolonged therapy, and increasing parasite resistance, underscoring the need for novel therapeutic approaches. Previous studies by our group demonstrated the antileishmanial activity of 3-phenyl-lawsone (3-PL) both *in vitro* and *in vivo*. In this study, we evaluated the activity of free 3-PL and 3-PL encapsulated in PLGA nanoparticles (3-PL-PLGA) against *L. braziliensis*. Cytotoxicity toward bone marrow-derived macrophages (BMDMs) was assessed using the MTT assay. For *in vitro* infection assays, BMDMs were infected with *L. braziliensis* (MOI 1:3) for 24 h/ 37°C/ 5% CO_2, and treated with varying concentrations of 3-PL or 3-PL-PLGA for 48 h. For *in vivo* experiments, hamsters were infected on the dorsum with 1×10^6 *L. braziliensis* parasites. Six weeks post-infection, animals received a single intralesional dose of Tween 80, Glucantime, 3-PL, or 3-PL-PLGA, followed by lesion monitoring for an additional 6 weeks. Neither 3-PL nor 3-PL-PLGA exhibited cytotoxicity up to 2,000 µM. *In vitro* assays demonstrated a significant reduction in the infection index starting at 80 µM for 3-PL and 8 µM for 3-PL-PLGA, with IC_{50} values of 25.64 µM and 7 µM, respectively. *In vivo*, a reduction in lesion size was observed, and parasitic load analysis via PCR is ongoing. These preliminary results demonstrate the antileishmanial activity of both formulations, with 3-PL-PLGA exhibiting approximately fourfold greater *in vitro* efficacy than free 3-PL, highlighting its potential for further preclinical development.
Supported by: CAPES, FAPERJ **Keywords:** Leishmania braziliensis;PLGA nanoparticle;naphtoquinone.

HP - 021 - Unraveling the modular evolution and expansion of the trypanosomatid DGF-1 protein family

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Large multigene families are a hallmark of trypanosomatid genomes and play major roles in genome plasticity and parasite adaptation. Among them, the Dispersed Gene Family 1 (DGF-1) of Trypanosoma cruzi remains one of the least understood because of its large size, repetitiveness, and fragmented annotations. Here, using a high-quality long-read assembly of T. cruzi, we performed comprehensive re-annotation and structural characterization of DGF-1. We identified seven tandemly repeated structural modules, here termed ribs, that form the extracellular region. This is followed by a distinct C-terminal membrane-associated region containing a conserved hat domain and multiple transmembrane helices. These domains are highly conserved across paralogous copies and the 7 ribs cluster according to positional identity rather than gene origin, indicating that the canonical seven-rib architecture predates the expansion of the family. DGF-1 proteins are present in the early-branching trypanosomatid species Paratrypanosoma confusum and several other trypanosomatids but absent in Leishmania and African trypanosomes, consistent with multiple independent secondary losses. Comparative analyses across Euglenozoa indicate that the DGF-1 architecture did not originate in free-living bodonids. Instead, Bodo saltans contains some proteins with rib-like domains and others containing the membrane-associated region, whereas the first complete DGF-1 architecture appears in P. confusum. Phylogenetic and comparative genomic analyses indicate that the family subsequently underwent lineage-specific losses and independent expansions, the largest expansion occurring in T. cruzi. Together, these findings reconstruct the evolutionary emergence of the DGF-1 architecture from pre-existing structural modules and provide a framework for one of the largest and most enigmatic gene families in trypanosomatids.
Keywords: DGF-1 modular architecture;Trypanosomatidae;Genome evolution.

HP - 022 - Targeting the PEX5–PEX14 Interaction induces glycosomal dysfunction and bioenergetic deficits in Leishmania

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Leishmaniasis are neglected diseases endemic in 99 countries, causing approximately 1 million cutaneous and 30,000 visceral cases annually. The current treatments are limited by toxicity, variable efficacy and relapses. Glycosomes are essential trypanosomatid organelles containing glycolytic and other metabolic enzymes synthesized in the cytoplasm and imported by PEX proteins. The PEX5–PEX14 interaction forms a transient import pore and is a potential drug target. Compounds based on a 3D pharmacophore mimicking this interaction inhibited Trypanosoma spp. with low toxicity to mammalian cells. Given the similarity between Trypanosoma and Leishmania systems, we selected four compounds effective against Leishmania (Leishmania) amazonensis and L. (L.) infantum promastigotes and investigated their functional effects. In amastigotes, EC50 values ranged from 3.82 to 15.43 μM for L. (L.) infantum and from 1.4 ± 0.1 to 3.3 ± 0.4 μM for L. (L.) amazonensis. Immunofluorescence assays using L. (L.) amazonensis expressing glycosome-targeted GFP showed that DOL630, one of the selected compounds, impaired protein transport and cytoplasmic GFP accumulation. Treated parasites also exhibited increased circularity, and fewer elongated cells. Intracellular ATP decreased in DOL630 treated L. (L.) infantum and L. (L.) amazonensis, and pyruvate supplementation did not reverse this effect. Cellular respiration also declined immediately after compound addition. The reduced ATP-linked respiration and estimated oxidative phosphorylation flux indicate impaired mitochondrial energy production. Thus, DOL630 disrupts glycosomal protein compartmentalization and causes a marked bioenergetic deficit. These findings support interference with PEX5–PEX14-dependent import, although further studies must determine whether mitochondrial dysfunction results from glycosomal damage or an additional compound effect. **Supported by:** FAPESP 2025/00260-0 **Keywords:** PEX14–PEX5 inhibitors;Glycosome;Drugs.

HP - 023 - NANOSTRUCTURED LIPID CARRIERS ENHANCE THE ANTILEISHMANIAL ACTIVITY OF Copaifera reticulata OIL AGAINST Leishmania (L.) amazonensis AMASTIGOTES

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Cutaneous leishmaniasis (CL) remains a public health challenge due to the limited efficacy and high toxicity of current treatments. Natural amazonian products, such as copaiba oil (CO), extracted from *Copaifera reticulata*, show promising antileishmanial activity. Encapsulating CO in nanostructured lipid carriers (NLCs) may improve its therapeutic performance. This study aimed to evaluate the *in vitro* antileishmanial activity of copaiba oil-loaded NLCs (NLC-CO) against intracellular *Leishmania (L.) amazonensis* and to characterize its interaction with infected macrophages. NLC-CO (2% CO v/v) and unloaded vehicle NLCs (NLC-V) were produced by hot homogenization and characterized using scanning and transmission electron microscopy (SEM/TEM). Cytotoxicity was evaluated in RAW 264.7 macrophages by MTT assay. Antileishmanial activity was assessed by infecting macrophages with *L. (L.) amazonensis* promastigotes, followed by treatment with different concentrations (2.5-40µg/mL) of NLCs or CO. CO concentrations refer to free oil, whereas NLC-CO concentrations refer to the total formulation. Infection was quantified by Giemsa staining and counting the number of intracellular amastigotes per 100 cells. SEM with cell fracturing enabled visualization of intracellular amastigotes across groups. Both SEM and TEM confirmed the presence of homogeneous spherical nanoparticles ranging in size from 100-200nm. Treatment with NLC- CO significantly reduced the number of intracellular amastigotes, with an IC_{50} of 6.7µg/mL, compared with an IC_{50}>40µg/mL for free CO. The selectivity index was 7.69 for NLC-CO and <5 for CO. Giemsa staining revealed a lower percentage of intracellular parasites in NLC-CO-treated cells (23.1%) compared with CO-treated cells (69%). SEM analyses confirmed a reduced parasite count. These findings suggest that nanoencapsulation increases the antileishmanial activity of CO, indicating that NLC-CO is a promising treatment for CL. **Supported by:** CNPq 442814/2025-9 & CNPq PQ-C 310403/2025-1 **Keywords:** Copaiba oil;Cutaneous leishmaniasis;Nanostructured lipid carriers.

HP - 024 - Role of ISP3 in Cell Death in Leishmania (Leishmania) amazonensis Analyzed Using Knockout and Overexpressing Strains

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Leishmaniases constitute a major public health concern due to their high incidence, wide geographic distribution and limited therapeutic options. This scenario highlights the need to identify new molecular targets in the parasite. Cell death in *Leishmania* has been associated with metacaspase (MCA), a cysteine protease with trypsin-like activity that is activated in response to different types of stress and drugs. Evidence obtained by our research group suggests that MCA activity may be negatively regulated by ISP3, a serine protease inhibitor expressed by the parasite. In this study, we generated *Leishmania (Leishmania) amazonensis* ISP3 knockout strains using CRISPR-Cas9. The knockout strains were validated by PCR and RT-PCR. At the current stage, an episomal expression vector is being constructed to reintroduce ISP3 into the knockout strains and generate ISP3-overexpressing lines. The mutant lines will enable the evaluation of the role of ISP3 in parasite viability, metacaspase activity, sensitivity to physical and chemical stress, as well as infectivity and survival in *in vitro* and *in vivo* in mouse infection models. Elucidating this mechanism may contribute to the identification of new therapeutic targets, especially considering that metacaspases are absent in vertebrates. **Supported by:** CAPES PROEX 88887.251956/2026-00 **Keywords:** Leishmania (Leishmania) amazonensis;ISP3;CRISPR-Cas9.

HP - 025 - Estudo da AMPK de *Rhodnius prolixus* durante a infecção por *Trypanosoma cruzi*

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A doença de Chagas é causada pelo *Trypanosoma cruzi*, cujo ciclo envolve hospedeiros vertebrado e invertebrado, sendo *Rhodnius prolixus* um de seus principais vetores. O parasito não possui todas as enzimas necessárias à síntese de lipídios, essenciais à sua diferenciação e proliferação, e modula o metabolismo dos hospedeiros para obtê-los. A proteína quinase ativada por AMP (AMPK) regula a homeostase energética dos eucariotos. Quando ativada (por fosforilação), a AMPK inibe processos anabólicos, como síntese de lipídios, e estimula processos catabólicos, como lipólise. O objetivo deste trabalho foi identificar ortólogos de AMPK em *R. prolixus* e entender seu funcionamento após alimentação e durante infecção por *T. cruzi*. Foi realizado BLASTp com sequências das subunidades α, β e γ da AMPK de *Homo sapiens* contra o genoma de *R. prolixus*, buscando sequências ortólogas em outras espécies de insetos. Pela análise dos domínios conservados, foram identificados 2 genes: *RPRC012957* (subunidade α) e *RPRC002957* (subunidade γ). A expressão gênica da subunidade α no corpo gorduroso (CG) e ovário (OV) foi maior nos dias 15 e 21 após alimentação, enquanto no músculo de voo (MV) o pico ocorreu no 10° dia. A subunidade γ apresentou maior expressão no CG nos dias 10 e 21, no MV no 21° dia e no OV nos dias 15 e 21. A expressão proteica da subunidade α, constitutiva e fosforilada, apresentou tendência ou maior expressão nos dias 3 e 10 após alimentação em todos os tecidos. A infecção por *T. cruzi* resultou em maior expressão do gene da subunidade α no 3° dia; entretanto, não alterou a expressão proteica de AMPK e pAMPK entre controles e infectados nos dias 3 e 10 no CG, MV e OV. O silenciamento de *RpAMPKα* foi eficiente e, preliminarmente, observou-se maior acúmulo de triacilglicerol nos insetos silenciados. Pela primeira vez, genes da AMPK foram identificados em *R. prolixus* e este trabalho auxilia no entendimento da enzima durante a infecção por *T.cruzi*. **Supported by:** FAPERJ, CAPES, CNPq
Keywords: Rhodnius prolixus;Trypanosoma cruzi;AMPK.

HP - 026 - Biochemical characterization of the D-serine transport system in *Trypanosoma cruzi* epimastigotes

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Trypanosoma cruzi, the causative agent of Chagas disease, relies on amino acids not only for protein synthesis but also as carbon and energy sources that support essential biological processes. The transport and metabolism of several L-amino acids have been extensively characterized. Among them, L-serine has recently emerged as a key metabolic substrate supporting parasite bioenergetics under nutritional stress. In contrast, the role of D-amino acids in trypanosomatids remains largely unexplored, with D-proline being the only exception. As *T. cruzi* has a putative serine racemase gene, we hypothesized that *T. cruzi* can transport D-serine as well, scavenging this D-amino acid from the hosts and enabling its metabolization. Here, we biochemically characterized D-serine transport in *T. cruzi* epimastigotes. D-serine uptake followed Michaelis-Menten kinetics (K_M = 0.47 ± 0.06 mM; V_{max} = 0.68 ± 0.02 nmol·min^{-1} per 10^7 cells), consistent with a low-affinity carrier-mediated transport system. Uptake depended on the transmembrane H^+ gradient and intracellular ATP levels. Competition assays showed that L-serine and L-alanine inhibited D-serine uptake by 98% and 90%, respectively. In contrast, D-alanine caused only 6% inhibition, whereas D-leucine, L-leucine, L-histidine, and L-proline did not inhibit D-serine uptake, indicating a specific but not stereoselective uptake system. Thermodynamic analyses yielded an Q_{10} of 2.03 and an activation energy of 54.28 ± 4.28 kJ·mol^{-1}, consistent with secondary active transport, and showed that transport activity increase between 10 °C and 45 °C, remaining stable across the physiological temperatures encountered by the parasite in the triatomine vector (25-28 °C) and inside the mammalian host (37 °C). Collectively, these results provide the first detailed biochemical characterization of a D-amino acid transport system in *T. cruzi*, paving the way for future studies on the physiological role of D-amino acids in parasite biology. **Supported by:** FAPESP: grant to ET N° 2025/27542-5, grant to MBA N° 2022/16078-8, and grant to AMS N° 2021/12938-0. **Keywords:** Trypanosoma cruzi;D-serine uptake;D-amino acids.

HP - 027 - In silico and in vitro characterisation of phosphatidylinositol 4-kinases from *Trypanosoma cruzi*: Evolutionary Origins, Implications for Cell Invasion, Molecular Modelling and Docking

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Phosphatidylinositol 4-phosphate (PI4P) is a phosphoinositide synthesised by phosphatidylinositol 4-kinases (PI4Ks). These are essential enzymes involved in cellular regulation, as well as being the main lipid determinants of the Golgi apparatus and trans-Golgi network. PI4K inhibitors have demonstrated therapeutic potential in the treatment of cancer, malaria and viral infections. However, the biological role of these kinases in *Trypanosoma cruzi* remains poorly understood. This study aimed to characterise *T. cruzi* PI4Ks using an integrated in vitro and in silico approach to investigate their function and therapeutic potential. Parasites from the G and Dm28c strains were transfected to express the PI4Kβ protein tagged with green fluorescent protein (GFP). The effects of protein localization on parasite growth, metacyclogenesis, and host cell invasion were then evaluated. Phylogenetic inference was performed using the maximum-likelihood method, while structural modelling and molecular docking were carried out using antimalarial and naphthyridine-derived inhibitors. PI4Kβ colocalised with AP-1 (Adaptor Protein 1) and cruzipain, indicating its presence in the Golgi complex, trans-Golgi network, reservosomes, and plasma membrane. Its expression did not significantly affect parasite proliferation or differentiation. The invasion rate remained unchanged in the Dm28c strain but increased in the metacyclic forms of the G strain under nutritional deprivation, suggesting a potential role for PI4Kβ in host cell invasion. Phylogenetic inference identified three monophyletic groups (PI3K, PI4Kα and PI4Kβ), indicating an ancestral duplication of the α and β isoforms, as well as high conservation of catalytic motifs. These findings emphasise the importance of PI4Ks as conserved and biologically relevant proteins, suggesting their potential as therapeutic targets for Chagas disease. **Supported by:** FAPEMIG, CNPq, CAPES **Keywords:** Chagas disease;PI4Kβ;Therapeutic target.

HP - 028 - Novel vaccine platform: TFR123 Enhances Cellular Immunity in a Chimeric mRNA Vaccine Against Trypanosoma cruzi

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Preclinical studies have identified mRNA-based vaccines as a promising approach for preventing Chagas disease (CD) and other protozoan infections. Here, we evaluated a lipid nanoparticle (LNP)-encapsulated mRNA vaccine encoding a chimeric antigen composed of epitopes derived from three Trypanosoma cruzi antigens, fused to the proprietary TFR123 vaccine platform (patent pending). TFR123 is designed to enhance antigen cross-presentation by antigen-presenting cells, thereby promoting efficient T-cell priming and cellular immunity. The antigenic sequence comprises selected epitopes derived from diferente *Trypanosoma cruzi* antigens. The resulting antigenic sequence was incorpored into the mRNA vaccine construct to investigate its ability to induce cellular and humoral immune response. After encapsulated into lipid nanoparticle, BALB/c mice were immunized with three doses of mRNA formulations using a prime-boost-boost protocol. Thirty days after the last immunization, blood and spleen samples were collected to evaluate humoral and cellular immune responses. As expected, neither the chimeric antigen alone nor the TFR123-containing construct induced detectable antibody responses. However, both formulations elicited significant IFN-γ production, with no differences between the groups. Notably, the TFR123-containing construct induced higher IL-10 levels than the chimeric antigen alone. These preliminary findings suggest that TFR123 represents a promising mRNA vaccine platform, inducing a Th1-type immune response associated with IL-10 production, which may contribute to a more balanced immune profile and reduced immunopathology during *T. cruzi* infection. **Supported by:** CNPq, CAPES, FAPEMIG, INCTV **Keywords:** mRNA vaccine;Chagas disease;Trans-sialidase.

HP - 029 - Analysis of long non-coding RNA CEBPB-AS1 regulating the transcription factor C/EBPβ in macrophages infected with Leishmania infantum

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Long non-coding RNAs (lncRNAs) are regulators of immune gene expression. CEBPB-AS1 is transcribed antisense from C/EBPβ gene and may regulate its expression. This transcription factor is a key regulator of macrophage responses, with activating (LAP/LAP*) and inhibitory (LIP) isoforms. During infection with *Leishmania infantum*, macrophages adopt a permissive state that supports parasite persistence. **We hypothesized that modulation of the CEBPB-AS1–C/EBPβ axis contributes to the macrophage state established during *L. infantum* infection.**
RNA-seq data from THP-1-derived macrophages infected with *L. infantum* for 24 h (PRJNA881925) were integrated with C/EBPβ ChIP-seq targets. Logistic regression assessed whether ChIP-associated genes were more likely to be differentially expressed. Candidate targets were then evaluated in an independent C/EBPβ-silencing dataset (GSE119707) and classified as positively regulated when expression decreased after knockdown or negatively regulated when it increased. Their behavior during infection was assessed by expression clustering and Gene Ontology enrichment.
C/EBPβ-associated genes showed an increased probability of differential expression during infection. Both positively and negatively regulated gene sets contained clusters induced by *L. infantum*, but with distinct immune signatures. Positively regulated genes were enriched in leukocyte chemotaxis and migration, whereas negatively regulated genes were associated with lymphocyte activation, mononuclear cell differentiation, and regulation of inflammatory responses. This bidirectional architecture is consistent with the dual transcriptional potential of C/EBPβ. Rather than driving a single macrophage activation program, C/EBPβ may coordinate immune recruitment while restraining selected inflammatory responses. This balance may contribute to a partially activated yet ineffective macrophage state that favors parasite persistence in visceral leishmaniasis.
Supported by: FAPESP: 2026/00534-5 **Keywords:** Long non-coding RNA;C/EBPβ;Leishmaniasis.

HP - 030 - Mitogenic stimulation as a strategy to generate genomic instability in *Trypanosoma cruzi*

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Trypanosoma cruzi is the etiological agent of Chagas disease, a neglected tropical disease that affects millions of people in Latin America. The first-line treatment is based on drugs developed several years ago that cause adverse effects and are frequently associated with the persistence of dormant drug-tolerant parasites. Therefore, novel therapeutic approaches are needed. This study investigates the genomic stability of *T. cruzi* following mitogenic stimulation based on the hypothesis that increased parasite proliferation enriches DNA damage accumulation, potentially sensitizing dormant parasites, to future combination therapies targeting the DNA damage response. Two mitogenic agents were evaluated: LB-100, a competitive protein phosphatase 2A (PP2A) inhibitor previously explored for similar purposes in cancer cells, and transforming growth factor alpha (TGF-α), a peptide reported to increase the replication rate of intracellular *T. cruzi* amastigotes. In silico analyses supported the potential interaction of LB-100 with the parasite PP2A. No significant differences regarding proliferation, viability, and cell cycle progression were observed following treatment with either mitogenic agent in *T. cruzi* epimastigote forms. However, intracellular amastigotes treated with 1 μM LB-100 exhibited an increased replication rate (p-value = 0.0073) accompanied by an accumulation of cells in the G1 phase of the cell cycle, suggesting possible activation of DNA damage-related checkpoints. These findings will be further investigated using TUNEL assays and EdU incorporation. Experiments with TGF-α in intracellular amastigotes are still in progress. Our preliminary results indicate that LB-100 acts as a mild stimulator of intracellular amastigote proliferation while altering cell cycle dynamics, supporting its potential use in subsequent studies aimed at inducing genomic instability in *T. cruzi* as part of a novel therapeutic strategy.
Supported by: FAPESP - 2024/07821-4 **Keywords:** Chagas disease;DNA damage;cell cycle.

HP - 031 - Drug repurposing: Teprenone modulates isoprenoid-dependent processes in Plasmodium falciparum and reduces the severity of experimental cerebral malaria

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Resistance of *Plasmodium* to antimalarial drugs has become a major challenge, supporting the search for new compounds, drug repurposing, and the development of effective therapeutic combinations. One of the most severe complications of malaria is cerebral malaria, a neurological syndrome associated with coma, various permanent sequelae, and potentially fatal outcomes. Based on this, we evaluated the *in vitro* and *in vivo* antiplasmodial potential of a synthetic terpene known as teprenone (geranylgeranylacetone) against *Plasmodium falciparum* and *Plasmodium berghei*. Our results demonstrated that *teprenone* exhibited *in vitro* antiplasmodial activity against the 3D7 strain, with an IC_{50} in the micromolar range. In addition, it exhibited low toxicity in HEP-G2 cells (human hepatocellular carcinoma) and a selectivity index greater than 10. Teprenone inhibited protein prenylation in *P. falciparum* in assays using radioactive FOH, reducing FPP incorporation into proteins, in addition to inhibiting GGOH transport in *P. falciparum* (3D7) cultures. In *in vivo* assays using a murine model of cerebral malaria, teprenone did not demonstrate efficacy in reducing parasitemia. Nevertheless, it had a beneficial effect in the treatment of cerebral malaria in C57BL/6 mice infected with *P. berghei* ANKA, increasing survival in the treated group compared with the control group. Treatment reduced IFN-γ levels in the brain, protected the blood–brain barrier from disruption, decreased leukocyte accumulation and the occurrence of microhemorrhages in the cerebral microvasculature. The results obtained in this study are expected to contribute to the development of new therapeutic strategies for cerebral malaria and encourage further investigations into the mechanism of action of teprenone.
Supported by: FAPESP grants 2017/22452-1 and 2024/09997-2, awarded to AMK. **Keywords:** cerebral malaria;prenylation of protein;geranylgeranylacetone.

HP - 032 - Naturally acquired anti-PvAMA1 antibodies compete with invasion-inhibitory monoclonal antibodies but do not appear to mediate protection against *Plasmodium vivax* recurrence in Amazonians

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Background. High levels of anti-apical membrane antigen 1 (AMA1) IgG during acute *Plasmodium vivax* infection in a Brazilian Amazon population were not associated with reduced risk of recurrence within 180 days. However, recombinant human monoclonal antibodies 826827 and 864865, targeting PvAMA1, inhibit *P. vivax* merozoite invasion of reticulocytes and sporozoite invasion of hepatocytes in vitro. We investigated whether naturally acquired anti-AMA1 antibodies compete with these monoclonal antibodies for binding to PvAMA1. **Objective.** To determine whether individuals from the *P. vivax*-endemic region of Mâncio Lima, Acre, Brazil, produce antibodies that compete with 826827 and 864865 for binding to PvAMA1. **Methods.** Pre-treatment plasma from patients enrolled in a randomized trial of chloroquine (CQ) alone or CQ-primaquine (PQ) for uncomplicated *P. vivax* malaria was tested in competition assays against the two monoclonal antibodies using the PvAMA1 ectodomain (residues 43–487), expressed in Pichia pastoris. Reactivity index and percentage inhibition of monoclonal antibody binding were assessed. Spearman correlations evaluated associations between reactivity and inhibition. Cox proportional hazards models assessed associations between inhibition terciles and recurrent malaria, stratified by sex and adjusted for age, treatment arm, and CYP2D6 genotype. The primary endpoint was first microscopy-confirmed recurrence between days 29 and 180 post-treatment. **Results.** Reactivity index and percent inhibition showed strong positive correlation for 826827 (Spearman $\rho = 0.63$, $p < 0.001$) and moderate positive correlation for 864865 ($\rho = 0.53$, $p < 0.001$). Naturally acquired antibodies showed comparable competition with both monoclonals, with marginally greater inhibition of 864865. Neither monoclonal antibody inhibition nor reactivity index was significantly associated with reduced risk of recurrence, while sex and age were stronger predictors of malaria risk. **Conclusion.** Naturally acquired anti-PvAMA1 antibodies competed with invasion-inhibitory monoclonal antibodies for PvAMA1 binding, suggesting recognition of similar, potentially overlapping epitopes. However, this competitive response was not associated with reduced risk of *P. vivax* recurrence. **Supported by:** Fundação de Amparo à Pesquisa do Estado de São Paulo, Brazil (FAPESP; 2024/04280-2 and 2025/07859-4) and Conselho Nacional de Desenvolvimento Científico e Tecnológico of Brazil (CNPq; 303039/2024-8). **Keywords:** Plasmodium vivax;monoclonal antibodies;apical membrane protein 1.

HP - 033 - B CELLS WITH A SENESCENCE-ASSOCIATED P16^{+}IL-8^{+} PHENOTYPE ACCUMULATE IN LOCALIZED CUTANEOUS LEISHMANIASIS LESIONS

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Localized cutaneous leishmaniasis (LCL), the most common clinical manifestation of leishmaniasis, is characterized by ulcerative skin lesions caused by Leishmania parasites transmitted through the bite of infected sandflies. Although the host immune response is essential for parasite control, excessive and persistent inflammation contributes to tissue damage and disease progression. Our group has previously demonstrated that senescent immune cells, particularly T and NK lymphocytes, accumulate within LCL lesions and are associated with poor clinical outcomes. Cellular senescence is characterized by irreversible cell cycle arrest and the acquisition of a senescence-associated secretory phenotype (SASP), which promotes chronic inflammation through the release of pro-inflammatory mediators, including IL-8. While B lymphocytes have traditionally been associated with immunomodulatory functions in LCL, mainly through IL-10 production, their involvement in cellular senescence remains poorly understood. In this study, indirect immunofluorescence was employed to characterize the senescente-associeted phenotype of B cells in LCL lesions. Compared with healthy skin, LCL lesions showed increased frenquencies of CD19^{+} B cells, p16^{+} cells and IL-8^{+} cells. Importantly, CD19^{+} B cells co-expressing the senescence marker p16 and IL-8 were also increased in lesions, indicating the presence of a B-cell population with a senescence-associeted and inflammatory phenotype. These findings expand the current understanding of B-cell biology in LCL by identifying p16+IL-8+ B cells as a previously unrecognized component of the lesional microenvironment. Further studies are warranted to elucidate their functional role in disease progression. **Supported by:** FAPES 51677.797.18389.30092022; CNPq 445530/2024-3 **Keywords:** B-cell population;Localized Cutaneous Leishmaniasis;senescence.

HP - 034 - Unraveling TcrPDEC-associated pathways in Trypanosoma cruzi mammalian stages: insights from transcriptomic and co-expression network analyses

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Chagas disease is a neglected tropical disease caused by *Trypanosoma cruzi*. Currently, only two treatments are available, both associated with significant toxicity that frequently leads to treatment discontinuation, highlighting the need to identify novel therapeutic targets. Cyclic AMP (cAMP) signaling plays a central role in parasite adaptation to changing environmental conditions throughout its life cycle. Among the enzymes regulating this pathway, *T. cruzi* phosphodiesterase C (TcrPDEC), which has been implicated in osmoregulation, was identified by our group as a promising therapeutic target. Since adaptation to the intracellular environment is essential for parasite survival and may affect the expression of potential drug targets, transcriptomic analyses were performed comparing axenic and intracellular amastigotes to identify molecular changes associated with this transition. Although TcrPDEC transcripts were detected under all analyzed conditions, their abundance was reduced by approximately 25% in intracellular amastigotes compared with axenic amastigotes and trypomastigotes, regardless of the host cell line analyzed, suggesting that TcrPDEC down-regulation is associated with intracellular adaptation. Gene co-expression network analysis identified a module containing TcrPDEC and 135 additional genes with highly coordinated expression patterns. Functional annotation revealed limited enrichment of biological functions, with only a small subset of genes associated with serine-type carboxypeptidase activity. These results suggest that TcrPDEC and genes within the same co-expression module may be regulated by common mechanisms; however, the biological pathways associated with this network remain to be elucidated. Ongoing proteomic analyses will provide further insights. Overall, these findings expand the current understanding of TcrPDEC regulation during amastigote adaptation and support its further investigation as a potential therapeutic target against *T. cruzi*. **Supported by:** **Keywords:** Transcriptomic;cAMP signaling;therapeutic target.

HP - 035 - Evaluation of the in vitro activity of the flavonoid quercetin against *Trypanosoma cruzi*

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Chagas disease, caused by the flagellated protozoan *Trypanosoma cruzi*, is one of the most important parasitic diseases in Latin America and is associated with significant morbidity, particularly in Brazil. Current treatment is limited to benznidazole and nifurtimox, which are effective primarily during the acute phase but exhibit limited efficacy during the chronic phase. Previous studies by our group demonstrated that quercetin possesses in vitro and in vivo activity against *Leishmania braziliensis*, a parasite belonging to the same family (Trypanosomatidae) as *T. cruzi*. Therefore, this study aimed to investigate the antiparasitic effects of the flavonoid quercetin against *T. cruzi* in vitro. Trypomastigotes (Y strain) were incubated with quercetin (0–300 µM) at 37 °C and 5% CO_2 and quantified daily using a Neubauer chamber over 24 to 48 hours. Cytotoxicity against mammalian cell lines was evaluated via the MTT reduction assay. To assess activity against intracellular amastigotes, monolayers of peritoneal macrophages from Swiss mice were infected with blood-derived trypomastigotes (1 parasite/cell) and treated with quercetin (0–330 µM) for 48 hours under the same incubation conditions. Quercetin significantly reduced trypomastigote motility starting at 37 µM, with an estimated IC50 of 77 µM. No cytotoxicity was observed in VERO cells or murine peritoneal macrophages up to 1320 µM. Against intracellular amastigotes, quercetin reduced the infection index, yielding an estimated IC_{50} of 160 µM. Additionally, no significant changes in macrophage nitric oxide production were observed. These results demonstrate that quercetin exhibits anti-*Trypanosoma cruzi* activity combined with low cytotoxicity in mammalian cells and an in vivo pilot study is underway to evaluate quercetin during the acute phase of infection. **Supported by:** FAPERJ e CAPES **Keywords:** Trypanosoma cruzi;Flavonoid; Quercetin.

HP - 036 - Robust T-cell dependent protection conferred by live-attenuated *Trypanosoma cruzi* deficient in active *trans*-sialidases

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Active *trans*-sialidases (aTS) are major virulence factors of *Trypanosoma cruzi*, involved in parasite infectivity and immune evasion. To investigate aTS role in host-parasite interactions and their potential as a vaccine target, we characterized immune responses elicited by CRISPR/Cas9-generated aTS knockout (aTS-KØ) parasites and investigated the contribution of $CD8^+$ T and B cells to aTS-KØ-induced protection. BALB/c and C57BL/6 mice were infected with wild-type (WT), aTS-KØ, or its addback strain (aTS-KØ AB), generated by CRISPR/Cas9-mediated restoration of the disrupted aTS coding sequence. Infection with aTS-KØ resulted in undetectable parasitemia, while aTS-KØ AB partially restored the phenotype. Parasite-specific IgG responses differed between mouse strains, as aTS-KØ induced lower IgG levels than WT in BALB/c mice but comparable responses in C57BL/6. In both strains, IgG responses against aTS in WT-infected mice were mainly directed toward its SAPA domain. Although cellular responses to parasite lysate were preserved, aTS-KØ infection reduced IFN-γ production against an epitope present exclusively in aTS, without affecting responses to an epitope from inactive *trans*-sialidases (iTS). Similar to BALB/c, C57BL/6 immunization with aTS-KØ induced parasite-specific IgG responses, including IgG1 and predominantly IgG2c, together with IFN-γ and IL-10, and conferred full protection against challenge with the virulent Y strain, also preserving body weight and improving survival. Importantly, protection was maintained in B cell-deficient ($\mu MT^{-/-}$) mice, which exhibited undetectable parasitemia after challenge, but was partially lost in $CD8^+$ T cell-deficient ($\beta 2m^{-/-}$) mice, showing that the protective immune response is T-cell dependent. Together, these findings confirmed the activity of aTS as an important virulence factor for *T. cruzi* and further supports the potential of aTS-KØ as a promising live-attenuated vaccine candidate for Chagas disease. **Supported by:** CAPES (88887.999804/2024-00); CNPq; FAPEMIG; INCTV **Keywords:** active trans-sialidases (aTS);live-attenuated vaccine;protective immunity.

HP - 037 - Role of aminoethylphosphonate and ethanolamine phosphate pathways in Trypanosoma cruzi

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The surface of *Trypanosoma cruzi* is coated with glycoconjugates, including glycosylinositolphospholipids (GIPLs) containing 2-aminoethylphosphonate (AEP), an unusual phosphonate. AEP metabolism is initiated by 2-aminoethylphosphonate pyruvate transaminase (2AEPPT), which interconverts AEP and pyruvate into phosphonoacetaldehyde and L-alanine. AEP and phosphoethanolamine (P-Etn) are components of *T. cruzi* GPI anchors and GIPLs, making the metabolic pathways related to these compounds particularly relevant to the parasite surface architecture. The biological role of 2AEPPT had not been established yet. Here we report the functional characterization of Tc2AEPPT. Recombinant Tc2AEPPT was produced and its enzymatic activity was experimentally confirmed . CRISPR-Cas9-mediated homozygous knockout ($^{-/-}$2AEPPT) epimastigotes showed a proliferation defect manifested as a 60% reduction of cell density at the stationary-phase, together with increased cell death. Was constructed an ethanolamine kinase (EtK) heterozygous knockout on $^{-/-}$2AEPPT background ($^{-/-}$2AEPPT/$^{-/+}$EtK). The $^{-/-}$2AEPPT/$^{-/+}$EtK clones also displayed reduced proliferation, suggesting that these enzymes affected both pathways contributing to GPI and GIPL biosynthesis. Exogenous AEP supplementation and episomal re-expression restored proliferation defects. Following severe nutritional stress, supplementation with AEP or P-Etn (as sole exogenous carbon sources) promoted recovery in both mutant lineages, supporting the importance of these metabolites for parasite survival and proliferation under nutrient limitation. Respiration assays revealed impaired mitochondrial bioenergetics in $^{-/-}$2AEPPT, with reduced routine respiration, electron transfer capacity, and spare respiratory capacity. Metacyclogenesis was significantly impaired. *In vitro* infection assays show that host-cell invasion was unaffected, but trypomastigote release was reduced, indicating a role for 2AEPPT in post-invasion stages of the intracellular developmental cycle. Together, these findings establish 2AEPPT as an important metabolic node linking phosphonate metabolism to parasite proliferation, bioenergetics, cell death, and infectivity. Due to its absence in mammals, 2AEPPT is a promising candidate for antiparasitic drug development. **Supported by:** FAPESP 2023/06791-1 **Keywords:** 2-aminoethylphosphonate pyruvate transaminase;Aminoethylphosphonate;Phosphoethanolamine.

HP - 038 - Establishment of an experimental replication model for the study of DNA replication in Trypanosoma cruzi amastigote form

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Trypanosoma cruzi is the causative agent of Chagas disease. Its life cycle involves the epimastigote and amastigote as non-infective and replicative forms, as well as the trypomastigote as infective and non-replicative forms. In this context, DNA replication is a fundamental mechanism for parasite biology, with evidence that this process is involved in the genomic plasticity of multigene families encoding virulence factors. Nevertheless, this area remains poorly understood, and most studies are conducted using the epimastigote forms, which is found inside the insect vector. The present study aims to better understand the replication of the amastigote forms. For that, we are establishing a model that mimics the conditions of patients with Chronic Chagas Cardiomyopathy (CCC) by performing an in vitro infection assay using fluorescent T. cruzi parasites (TcCLB LucNG) and a cardiomyocyte cell line with rare pathological genetic variant that have been shown to be linked with CCC in the presence or absence of inflammatory cytokines. The cardiomyocyte cells were infected with TcCLB LucNG using multiplicity of infections 10:1; 20:1. Then, samples were evaluated after different time points (24h and 48h) using fluorescence microscopy and/ or Operetta CLS high content analysis system (Revvity). We obseved a lower percentage of infection in cells treated with cytokines in every analyzed condition. Furthermore, cytokine-treated groups showed higher amastigote proliferation, particularly with IFN-γ, indicating that this treatment might modulate the intracellular environment in a way that favors T.cruzi proliferation. These results will be confirmed by the incorporation of thymidine analogs into T. cruzi amastigote forms to determine if there are changes in the cell replication rate at this modulated intracellular environment. **Supported by:** 2026/02991-4 **Keywords:** Human cardiomyocytes;Proliferation assay;Pathological Variants.

HP - 039 - LABCG1 Affects *Leishmania (Leishmania) amazonensis* Infectivity but Not Virulence or Sand Fly Infection

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Leishmaniases are diseases caused by protozoan parasites of the genus *Leishmania*. They can be classified into two main clinical forms: tegumentary and visceral leishmaniasis (TL and VL, respectively). Our group has studied the LV79 and PH8 strains of *Leishmania (Leishmania) amazonensis*, one of the species predominantly causing TL in Brazil. PH8 is more infective *in vitro* and more virulent *in vivo* compared to LV79. Proteomic analysis of promastigotes from both strains revealed that the ABCG1 transporter is 23-fold more abundant in the PH8 than in the LV79 strain. To determine whether *L. (L.) amazonensis* ABCG1 contributes to the higher virulence of the PH8 strain, we used a CRISPR-Cas9-based strategy to generate a knockout line for this gene. The mutants were compared to wild-type parasites regarding growth, metacyclogenesis, LPG characteristics, resistance to complement-mediated lysis, *in vitro* infectivity, *in vivo* virulence, and their ability to establish themselves in the sand fly vector. Overall, we demonstrate that while the loss of LABCG1 did not impact the growth, metacyclogenesis, LPG characteristics, or resistance to complement-mediated lysis of *Leishmania (Leishmania) amazonensis* parasites, it did affect parasite infectivity *in vitro*, but not *in vivo*, both in the mouse model and in the sand fly vector. These findings indicate that LABCG1 contributes to *L. (L.) amazonensis* infectivity but is not essential for parasite survival and virulence *in vivo*. **Supported by:** FAPESP 2021/08666-4, 2021/08915-4; PROEX 88887.623653/2021-00; Intramural Research Program of the National Institutes of Health (NIH) **Keywords:** Leishmania (Leishmania) amazonensis;ABCG1;CRISPR-Cas9.

HP - 040 - Characterization of the NEDD8 Pathway in Leishmania infantum: Functional and Biochemical Insights

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Leishmania infantum is the causative agent of visceral leishmaniasis (VL) in Brazil, the most severe form of the disease, which can be fatal in up to 95% of untreated cases. As leishmaniasis remains a major global health concern, understanding the molecular mechanisms underlying parasite survival and adaptation is essential. These processes are regulated by post-translational modifications that modulate protein activity, stability, and cellular pathways. Among these modifications, protein neddylation—the covalent conjugation of the ubiquitin-like protein NEDD8 to target substrates—plays a key role in regulating Cullin–RING E3 ubiquitin ligase (CRL) activity and cell cycle progression in eukaryotic cells. However, the repertoire of neddylated substrates and their functional roles in *L. infantum* remain largely unknown. In this study, we performed a functional and biochemical characterization of LinfNEDD8 by investigating its interacting partners and their potential roles in parasite viability, proliferation, and cell cycle progression. Mass spectrometry analysis of the LinfNEDD8 interactome revealed proteins involved in multiple intracellular processes, including eight Cullin proteins and key components of the neddylation machinery, such as E1 and E2 enzymes. We validated the neddylation of LinfCul1 using a cell line co-expressing myc-mCherry-LinfCul1 and HA-LinfNEDD8, followed by co-immunoprecipitation assays. Finally, parasite viability was evaluated following treatment with MLN4924, a clinically relevant inhibitor of the human neddylation pathway. No significant effect on parasite viability was observed, suggesting that the neddylation system in *L. infantum* may differ from that of *Homo sapiens*. Ongoing studies are focusing on the biochemical characterization of the neddylation system, including the recombinant expression and purification of E1 and E2 enzymes, to evaluate the regulation of neddylation by inhibitors of the human neddylation pathway. In addition, the E2 interactome will be investigated to further elucidate the components of the neddylation pathway in *L. infantum*. Together, these findings provide new insights into the neddylation pathway in *L. infantum* and highlight its potential as a target for the development of novel antiparasitic therapies.
Supported by: FAPESP: 2025/16692-6 **Keywords:** Leishmania infantum;Neddylation ;Characterization.

HP - 041 - Cardiac microarchitecture in acute Trypanosoma cruzi infection: highlights from synchrotron X-ray microtomography

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Chagas disease, caused by Trypanosoma cruzi, begins with an acute parasitemic phase and typically progresses to a chronic phase, during which most individuals remain asymptomatic, while a subset develops Chagas cardiomyopathy. Although Chagas cardiomyopathy becomes clinically relevant primarily during the chronic phase, parasitic and inflammatory alterations can already be detected in the myocardium during acute infection. Conventional two-dimensional methods, however, limit assessment of their spatial organization within the tissue. Here, we investigated cardiac tissue during acute T. cruzi infection using synchrotron X-ray microtomography at the MOGNO beamline of Sirius (LNLS/CNPEM, Brazil). In addition, a machine learning-based tool is currently under development to assist in the identification and characterization of infection-associated cardiac alterations in the tomographic datasets. Female BALB/c mice (n = 10) were inoculated intraperitoneally with 105 bloodstream trypomastigotes of the Dm28c strain, while uninfected animals served as controls (n = 5). On day 20 post-infection, animals were euthanized, and the hearts were perfused and fixed to preserve tissue architecture. Following sectioning, dehydration, PTA staining, and paraffin embedding. The 68 samples were prepared, of which 44 were analyzed, and 20 were selected for zoom microtomography. Preliminary data for three-dimensional reconstructions enabled the identification of amastigote nests within the cardiac tissue of infected animals. These parasitic foci were observed in regions associated with myocardial fiber disorganization, whereas control animals showed preserved cardiac architecture. These findings highlight the potential usefulness of this technique combined with computational image analysis to investigate acute cardiac pathogenesis and provide a basis for future investigations into mechanisms of tissue damage and the development of therapeutic strategies. **Supported by:** FINEP Grant #01.22.0473.00 **Keywords:** Chagas disease;X-ray microtomography;Imaging reconstruction.

HP - 042 - A Therapeutic RNA vaccine based on *Trypanosoma cruzi* Trans-sialidase protects mice in a chronic model of Chagas disease

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Chagas disease (CD) remains a serious public health problem, with high morbidity rates and global economic impact. Although effective during the acute phase, Benznidazole (BZ) treatment during the chronic phase has low success rates and causes severe adverse effects. Different studies towards vaccine development have used Trans-sialidase (TS), a key *Trypanosoma cruzi* surface protein involved in immune evasion and parasite survival, as a target antigen. Our group showed that mice immunization with mRNA of TS induces a protective Th1 response that is efficient in a prophylactic immunization protocol. After developing a chronic model of infection in BALB/c mice infected with a Y strain expressing luciferase, we tested a therapeutic protocol consisting of TS mRNA encapsulated into lipid nanoparticle (LNP). Efficacy was assessed through evaluation of parasitemia, survival rates, bioluminescence, cardiac/gastrointestinal histology and immunological analyses. The chronic murine model of infection mimics the cardiac/intestinal alterations of CD. Infected mice were treated with TS mRNA/LNP using a four-dose protocol with or without association with a suboptimal dose of BZ. Both therapeutic protocols induced specific humoral responses anti-TS and increased production of IFN-γ, IL-10, and IL-4. Assessment of cardiac function by electrocardiogram (ECG) recordings showed that treatment with TS mRNA/LNP improved the ECG score compared with the untreated group. Finally, histopathological analyses of the heart and colon of mice vaccinated with TS mRNA/LNP showed a significant reduction in inflammatory infiltrate and tissue and neuronal protection when compared to infected mice. Collectively, these findings showed that TS mRNA vaccination is a promising therapeutic strategy against CD, inducing a balanced immune response, with reduced tissue inflammation as well as cardiac and intestinal dysfunctions that are associated with chronic infection. **Supported by:** Capes, CNPq, FAPEMIG, INCTV **Keywords:** mRNA vaccine;Trans-sialidase;Chagas disease.

HP - 043 - Discrete typing unit genetic classification of Trypanosoma cruzi present in different hosts of a Brazilian Amazonian region, revealed different wild and peridomestic parasite cycle

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Chagas disease (CD) or American trypanosomiasis is a zoonosis caused by the protozoan Trypanosoma cruzi (T. cruzi). Infection with T. cruzi can occur through vector-borne transmission by triatomine insects, through contaminated blood and derivatives, congenitally and by ingestion of infected triatomines. After the initial infection, an acute phase with severe symptoms may occur, but commonly, CD evolves to a chronic or indeterminate phase, characterized by the absence of clinical signs and symptoms, over time, may cause symptoms of cardiac or digestive involvement, associated with the different genetic populations of T. cruzi. According to the most recent classification, there are six genetic populations of T. cruzi (discrete typing units – DTUs, TcI to VI), distributed according to the region and habits of the transmitting triatomines. Knowledge of these populations is important for planning actions to control the transmission of T. cruzi. The diagnosis of CD by serological tests uses the parasite as an antigen, which reduces the specificity of the method. In the chronic phase, detection of parasite nucleic acids can be performed by nucleic acid detection methods. The objective of this study was to investigate which DTUs are present across different ecotopes in an endemic region of northern Brazil, directly from biological samples of human and animal populations, and triatomines. The results revealed the presence of T. cruzi from DTU I in triatomines present in palm trees and another T. cruzi DTU was observed in the human, and peridomestic/domestic animal populations. Investigation of triatomine blood feeding source showed it was from wild animal species. This research revealed two different T. cruzi cycles: one among palm tree triatomines and wild animals, and the other including domestic/peridomestic animals, human population, and an unknown vector. These results reinforce the importance of T. cruzi genetic surveillance in infection risk areas. **Supported by:** Fundação de Amparo a Pesquisa - FAPESP **Keywords:** Trypanosoma cruzi;Discrete Typing Units;molecular diagnostics.

HP - 044 - Dermal Fibroblasts Display Senescence-Associated and Pro-Inflammatory Features in Cutaneous Leishmaniasis

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Cutaneous leishmaniasis (CL) caused by Leishmania braziliensis is characterized by intense and persistent inflammation that contributes to tissue damage. Although immune-mediated mechanisms of CL pathology have been extensively investigated, the contribution of stromal cells to the lesional inflammatory microenvironment remains poorly understood. Fibroblasts can undergo cellular senescence and acquire a senescence-associated secretory phenotype capable of sustaining tissue inflammation. Here, we investigated whether fibroblasts from CL patients exhibit senescence-associated and inflammatory features. Dermal fibroblasts derived from lesions of CL patients and healthy controls were assessed for senescence-associated β-galactosidase (SA-β-gal) activity and expression of γH2AX and p21. CL-derived fibroblasts showed increased SA-β-gal activity and higher frequencies of $\gamma H2AX^+$ and $p21^+$ cells compared with healthy controls. Importantly, fibroblast populations displaying senescence-associated features, characterised by FAP, FSP1 or α-SMA expression together with γH2AX, p21 or p38 positivity and absence of Ki-67, were also identified within CL lesions. Functionally, CL-derived fibroblasts exhibited increased IL-6 secretion, whereas MCP-1 levels were unchanged. Consistently, an increased frequency of $Vimentin^+IL\text{-}6^+$ cells and $Vimentin^+IL\text{-}8^+$ cells was observed in CL lesions compared with healthy skin. Together, these findings reveal that fibroblasts in CL acquire a persistent senescence-associated and inflammatory phenotype that is maintained ex vivo and mirrored within the lesional tissue. This stromal phenotype may contribute to sustaining the inflammatory microenvironment associated with CL immunopathology. **Supported by:** CNPq: 445530/2024-3 **Keywords:** cutaneous leishmaniasis ;cellular senescence;fibroblasts .

HP - 045 - AUTOPHAGY IN TRYPANOSOMA CRUZI DURING OXIDATIVE STRESS CONDITIONS: IMPLICATIONS FOR MITOCHONDRIAL REMODELING

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Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, affects millions of people worldwide, particularly in the Americas. *T. cruzi* undergoes a complex life cycle and is continuously exposed to stressful conditions, requiring morphological and metabolic flexibility. The parasite mitochondrion undergoes intense morphological and functional remodeling throughout its life cycle, although the molecular mechanisms underlying these processes remain poorly understood. Autophagy is a major pathway involved in the degradation and recycling of damaged proteins and organelles, contributing to cellular homeostasis and adaptation. Here, we aimed to investigate the role of autophagy in mitochondrial remodeling in *T. cruzi* under different stress conditions. Using CRISPR/Cas9, we generated CL Brener epimastigote mutants lacking both copies of *ATG3*. ATG3 disruption impaired parasite growth by up to 47.2% during the logarithmic phase compared with wild-type cells. Interestingly, transcripts of several autophagy-related genes, such as *VPS15*, *VPS34*, *ATG6*, *ATG9*, *ATG4.1*, *ATG4.2*, *ATG7*, *ATG8.1*, and *ATG8.2*, were up to 4.2-fold higher in ΔATG3 mutants. Despite these changes, MET and MEV analyses revealed no apparent morphological differences between wild-type and mutant cells. *ATG3* disruption altered the response to mitochondrial stress, with ΔATG3 mutants showing increased resistance to oligomycin (IC_{50}/24h = 19.5 µM vs. 152.4 µM) but not antimycin A. Both treatments reduced mitochondrial membrane potential (ΔΨm) in both strains. ROS analysis revealed that antimycin A increased ROS levels in both strains, whereas oligomycin specifically increased ROS in ΔATG3 mutants. These findings suggest that ATG3-mediated autophagy contributes to parasite adaptation to mitochondrial stress and organelle homeostasis. Further studies will assess its potential as a target to disrupt *T. cruzi* adaptation throughout its life cycle. **Supported by:** FIOCRUZ, CNPq, CAPES e FAPERJ **Keywords:** Trypanosoma cruzi;Autophagy;Mitochondria.

HP - 046 - Study of Leishmania (Viannia) lainsoni clinical isolates from North Brazil

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Leishmaniases are parasitic diseases caused by protozoans of the genus *Leishmania*. These diseases are widely distributed worldwide and are highly prevalent in Brazil. Tegumentary leishmaniasis (TL) represents an important public health concern, particularly affecting socioeconomically vulnerable populations. In Brazil, the Northern region is characterized by a high diversity of dermotropic *Leishmania (Viannia)* species. Among them, *L. (V.) lainsoni* stands out for causing a mild cutaneous disease in humans and for its genetic discrepancy compared to other *Viannia* species. Herein, we report biological traits of six *L. (V.) lainsoni* clinical isolates from patients with localized cutaneous leishmaniasis treated at the Evandro Chagas Institute (IEC), Pará, Brazil. These patients, with a disease duration of up to two months, presented with lesions showing mild ulceration and were treated with two courses of pentavalent antimonial therapy. We used PCR-RFLP targeting the RNA polymerase II gene to confirm all isolates as *L. (V.) lainsoni*. We also showed that none of the isolates has evidence of *Leishmania* RNA Virus I (LRV1). Their in vitro growth dynamics were evaluated daily using a Neubauer chamber and compared with strains of *L. (V.) lainsoni* M6426 and *L. (V.) braziliensis* M2903. We observed a variability in in vitro growth among the clinical isolates, with most isolates reaching the stationary phase by day 5. The highest parasite density was approximately 5×10^7 promastigotes/mL and the lowest less than 2.5×10^7 promastigotes/mL. The next steps of our study are to evaluate the in vitro infectivity of the isolates in bone marrow-derived macrophages, their complement susceptibility and their virulence in BALB/c mice. Our findings will contribute to expanding the knowledge of the biology of *L. (V.) lainsoni*. **Supported by:** FAPESP 2026/00013-5; CAPES 88887.254327/2026-00. **Keywords:** Leishmania (Viannia) lainsoni;Tegumentary leishmaniasis;Clinical isolates.

HP - 047 - Functional analysis of the E3 ubiquitin ligase LinfHrd1: a pontential component of Endoplasmic Reticulum-Associated Degradation in *Leishmania infantum*

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Endoplasmic Reticulum-Associated Degradation (ERAD) is a eukaryotic pathway that removes misfolded proteins from the endoplasmic reticulum (ER) for proteasomal degradation. The E3 ubiquitin ligase Hrd1 is a central component mediating substrate retrotranslocation and ubiquitination. ERAD plays an essential role in maintaining the homeostasis of protein folding. This process is particularly important in organisms that alternate hosts, such as protozoan parasites of the genus *Leishmania*, which alternate between extracellular promastigotes in the invertebrate host and intracellular amastigotes in mammalian macrophages, facing changes in temperature, pH, and oxidative stress levels. However, the ERAD pathway remains unexplored in *Leishmania spp.. Leishmania infantum* encodes an Hrd1 ortholog (LinfHrd1) that shares 20% and 26% amino acid identity with *Saccharomyces cerevisiae* and *Homo sapiens* Hrd1, respectively. Structural analysis indicated that LinfHrd1 is similar to both orthologs, presenting RMSD values of 12 Å (human) and 8.5 Å (yeast). The transmembrane domain is conserved, whereas the RING domain shows divergence among organisms, contributing to the higher RMSD values. The LinfHRD1 gene knockout generated viable parasites with slower proliferation than the wild type. Notably, ΔLinfHRD1 cells were unable to differentiate into amastigotes or proliferate at 37 °C, suggesting their role in parasite adaptation to mammalian host conditions. In addition, cell cycle evaluation by flow cytometry revealed a significant reduction in the G0/G1 population in KO cells. Ongoing analyses include macrophage infection assays and the LinfHrd1 interactome obtained by immunoprecipitation followed by mass spectrometry. A biochemical characterization involving heterologous expression and purification of soluble LinfHrd1 for an in vitro ubiquitin charging assay with purified E2 enzymes LinfUbc7 and LinfUbc8 is being developed. These findings contribute to the characterization of the ERAD pathway in *L. infantum*, providing insights into its relevance to parasite biology and host adaptation. **Supported by:** Processo FAPESP 2024/17930-5 **Keywords:** Endoplasmic Reticulum-Associated Degradation;Hrd1;Leishmania infantum.

HP - 048 - Functional characterization of carbamoyl phosphate synthetase II in Trypanosoma cruzi

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Chagas disease remains a major global public health problem caused by *Trypanosoma cruzi*. Carbamoyl phosphate synthetase II (CPSII) and aspartate transcarbamoylase (ATCase) are the first two enzymes of the de novo pyrimidine biosynthesis pathway, which provides precursors for nucleic acid (DNA and RNA) synthesis. Beyond this knowledge, their biological functions in *T. cruzi* remain poorly understood. In this work, we investigated the role of CPSII in parasite metabolism, particularly amino acid metabolism, and its contribution to different aspects of the life cycle of *T. cruz*i. To investigate CPSII function, hemi-knockout parasites were obtained using a CRISPR/Cas9-mediated strategy. Parasite proliferation, metacyclogenesis, programmed cell death (PCD) and cell-cycle progression were assessed by proliferation assays and flow cytometry. CPSII hemi-knockout parasites exhibited 20–30% lower proliferation than the parental Cas9 line after five days of culture, with a significant reduction observed at the end of the proliferation assay (P < 0.0001). During metacyclogenesis, the parental Cas9 line reached 66–79% metacyclic trypomastigotes between days 5 and 8, whereas the hemi-knockout clones showed only 12–30% differentiation. Flow cytometry analysis demonstrated an increase in late PCD cells at day 8, from 5.1% in the parental Cas9 line to approximately 17% in all hemi-knockout clones. In contrast, no consistent alterations in cell-cycle progression were observed among the hemi-knockout parasites, although one clone showed a transient increase in the G1 population. Because hemi-knockout parasites still retained one functional CPSII allele, a SHARK-based conditional knockdown system is currently being established to further investigate CPSII essentiality and its role in parasite metabolism. This approach will enable a more precise characterization of CPSII function throughout the *T. cruzi* life cycle. Taken together, our findings demonstrate that partial disruption of CPSII compromises parasite proliferation, survival and differentiation without inducing a marked cell-cycle arrest. These results support an important role for CPSII in the biology of *T. cruzi* and provide a foundation for future studies exploring its metabolic function and potential as a therapeutic target. **Supported by:** FAPESP No. 2024/22343-1 No. 2021/12938-0 **Keywords:** Trypanosoma cruzi;Carbamoyl phosphate synthetase II;Metabolism.

HP - 049 - Miltefosine susceptibility of Leishmania infantum isolates from dogs with visceral leishmaniasis in the State of Sao Paulo

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Visceral leishmaniasis (VL), caused by *Leishmania infantum*, is a severe anthropozoonotic disease with high lethality, with domestic dogs (*Canis familiaris*) acting as the main domestic reservoir. In Brazil, miltefosine is the only drug approved for canine treatment. Although it promotes clinical improvement and reduces parasite load, it presents limitations regarding complete parasitological cure, leading to frequent relapses. Widespread miltefosine use raises concerns about selective pressure and the emergence of strains with reduced susceptibility, which could compromise future human treatment strategies and underscores the necessity of monitoring circulating isolates. This study aimed to evaluate the in vitro susceptibility profile to miltefosine of L. infantum isolates from VL-seropositive dogs. Eight clinical specimens (spleen or lymph node aspirates) were collected from naturally infected dogs in endemic regions of São Paulo State (2024–2025). Primary isolation was conducted in biphasic medium (BAB with rabbit blood + BHI with Schneider's medium and FBS), followed by parasite maintenance in M199 medium. Promastigotes were incubated with varying miltefosine concentrations for 48 h, and cell viability was evaluated via an MTT colorimetric assay. Among the eight isolates, the 50% Effective Concentration (EC50) values ranged from 8.5 to 22.0 µM, compared to 7.0 µM for the reference strain. Notably, one isolate was 3.14-fold less sensitive ($p < 0.05$) to miltefosine than the reference strain. These results demonstrate variable susceptibility profiles among circulating field isolates, highlighting the importance of continuous epidemiological surveillance. Future assays using intracellular amastigotes will be essential to further validate these findings. **Supported by:** FAPESP nº processo 2024/15388-9, 2025/27284-6, 2026/13612-4, CAPES #2022-0/88881.689557 PROAP-AUXPE #2022/115 **Keywords:** drug resistance;phenotypic assay;Visceral leismaniasis.

HP - 050 - In vitro susceptibility profile to amphotericin B of Leishmania infantum isolates from dogs in endemic regions

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Amphotericin B, a polyene macrolide antibiotic, is a cornerstone leishmanicidal drug widely used in the treatment of human visceral leishmaniasis (VL). Leishmania infantum is the primary etiological agent in the Americas and Mediterranean regions, and domestic dogs act as the main reservoir. Monitoring the drug susceptibility profile of circulating isolates in domestic reservoirs is critical to detect natural variability or emerging resistance that could compromise human therapeutic efficacy. This study aimed to evaluate the in vitro susceptibility profile to amphotericin B of L. infantum isolates obtained from VL-seropositive dogs. Five clinical specimens (spleen or lymph node aspirates) were collected from naturally infected, euthanized dogs in endemic regions of São Paulo State (2024–2025). Primary isolation was conducted in biphasic medium (BAB with rabbit blood + BHI with Schneider's medium and FBS), followed by parasite maintenance in M199 medium. Promastigotes were incubated with varying amphotericin B concentrations for 48 h, and cell viability was evaluated via MTT colorimetric assay. Among the five isolates, 50% Effective Concentration (EC50) values ranged from 47.8 to 67.0 nM, compared to 79.3 nM for the standard reference strain. Two isolates exhibited a 1.6-fold higher sensitivity ($p < 0.05$) to amphotericin B than the reference strain. These results demonstrate susceptibility variability among circulating field isolates without evidence of resistance. Continuous phenotypic monitoring in domestic reservoir populations is an fundamental strategy to detect emerging resistance and preserve the clinical longevity of amphotericin B. Future assays using intracellular amastigotes will further validate these findings. **Supported by:** FAPESP nº processo 2024/15388-9, 2025/27284-6, 2026/13612-4, CAPES #2022-0/88881.689557 **Keywords:** drug resistance,;phenotypic assay;visceral leishmaniasis.

HP - 051 - Investigating distinct immunization protocols based on Trans-sialidase mRNA and recombinant protein to develop a Chagas disease vaccine

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RNA vaccines have emerged as a promising strategy against Chagas disease. Trans-sialidases (TS) present on the surface of *Trypanosoma cruzi* are responsible for transferring sialic acid residues from host glycoconjugates to mucins on the parasite surface, a mechanism that is related to the parasite capacity to evade the host immune system. TS contains a C-terminal immunogenic domain with amino acids repeats known as SAPA (Shed Acute Phase Antigen). Previously, our group demonstrated that immunization with mRNA containing the repeat domain increased IL-10 production, which was associated with a more balanced inflammatory response. Therefore, we evaluated different immunization regimens using TS-encoding mRNA, comparing two- and three-dose schedules, as well as a heterologous strategy combining RNA and recombinant TS protein, with the aim of optimizing the vaccination regimen. The coding sequence of TS containing the SAPA domain was synthesized in a mammalian expression vector, which was subsequently used for in vitro mRNA transcription. The mRNA was transfected into HeLa cells, and TS antigen production was confirmed by Western blot. TS mRNA was then formulated with lipid nanoparticles (LNPs) for immunization. Recombinant TS protein was generated by cloning and heterologous expression in *Escherichia coli*. Immunization with two doses of TS mRNA/LNP induces a cellular immune response comparable to that elicited by three doses or the heterologous regimen, as well as similar protection against challenge with a virulent *T. cruzi* strain, as shown by parasitemia and tissue parasitism. However, immunization with two doses of TS mRNA/LNP resulted in reduced histopathological scores in the heart and colon, comparable to those observed following three doses of mRNA/LNP and lower than those observed with the heterologous RNA/protein regimen. These findings suggest that two doses of TS mRNA/LNP may be sufficient to induce a protective immune response against Chagas disease. **Supported by:** CNPq **Keywords:** Trans-sialidase;Chagas disease;mRNA.

HP - 052 - Impact of Trypanosoma cruzi extracellular vesicles on peritoneal macrophage infection

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There are currently 25 diseases listed as neglected tropical diseases, including Chagas disease (CD). CD is caused by the protozoan Trypanosoma cruzi, affecting more than 7 million people mainly in endemic areas of Latin America. Treatment is based on two nitroderivatives, benznidazole (Bz) and nifurtimox (Nf), which have several limitations, including severe adverse effects, lower effect at the later disease stages (chronic) besides the occurrence of naturally resistant strains. Therefore, searching for alternative therapies is necessary. Extracellular vesicles (EVs) are small cellular components surrounded by a lipid bilayer composed of bioactive molecules inherited from the cell of origin. Previous studies have reported that EVs released by T. cruzi modulate several host cell events. Thus, the main objective of the present study is to investigate the effect of EVs derived from different forms of T. cruzi on their infection on mammalian host cells. Therefore, were used. EVs were isolated from epimastigote and culture-derived trypomastigote forms of T. cruzi (Y strain) using the Total Exosome Isolation Reagent and characterized by transmission electron microscopy (TEM) and nanoparticle tracking analysis (NTA). Peritoneal mouse macrophages (PMM) were obtained by peritoneal lavage of male Swiss mice. The cells were plated and incubated with different concentrations of EVs derived from both parasite forms. After 24 h, PMM were rinsed using saline and then infected for 24 h with bloodstream trypomastigote forms of T. cruzi (Y strain) to evaluate the infectivity profile by light microscopy. Infected cultures incubated only with culture medium were used as controls. Our preliminary data suggest that prior incubation of mouse peritoneal macrophages with EVs derived from the different developmental forms of T. cruzi partially reduces the parasite burden. **Supported by:** CNPq, FAPERJ, PROEP e FIOCRUZ **Keywords:** Extracellular vesicles;Chagas disease;Trypanosoma cruzi.

HP - 053 - A Polyepitope Vaccine Candidate Promotes TH1-Associated Immunity and Controls Leishmania infantum Infection in Syrian Golden Hamsters

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Visceral leishmaniasis (VL), caused by *Leishmania infantum*, remains one of the most impactful neglected tropical diseases worldwide, being associated with high mortality, broad geographic distribution, and limitations in the currently available control strategies. The lack of a licensed human vaccine, together with the need for improved preventive strategies for dogs, the main domestic reservoir of the parasite, highlights the urgent need for safe and effective vaccines. This study aimed to evaluate the safety, immunogenicity, and protective efficacy of a polyepitope protein candidate (Chi-A) adjuvanted with Poly-ICLC (Hiltonol) as a potential human vaccine against VL. Male and female Syrian golden hamsters (*Mesocricetus auratus*) received three subcutaneous immunizations at 15-day intervals and challenged 21 days after the third dose with *L. infantum* promastigotes (MCAN/BR/2008/OP46 strain). Sixty days post-challenge, vaccine safety, immunogenicity, and protective efficacy were assessed using automated hematology analyzer, multiparameter flow cytometry and real-time PCR. Overall, erythrocyte and platelet profiles were preserved, with no relevant alterations among the experimental groups. Minor changes were observed in the leukocyte profile, including reduced granulocyte levels, relative lymphocytosis in the Chi-A/Hiltonol group, and relative monocytosis across all experimental groups. Immunization with Chi-A/Hiltonol increased the frequency of IFN-gamma-producing $CD4^+$ cells and reduced IL-10-producing $CD4^+$ cells, indicating a shift toward a T_H1-associated immune profile. All infected groups showed a significant increase in relative spleen weight compared with the non-infected control group, confirming infection-associated splenomegaly. Although no significant differences were detected among the infected groups, animals vaccinated with Chi-A exhibited a smaller size, which was also consistent with the macroscopic appearance of the spleens. Importantly, immunized hamsters exhibited a marked reduction in splenic parasite burden, supporting the protective potential of the evaluated vaccine candidate. Taken together, these findings support the immunogenic and protective potential of Chi-A against *L. infantum*, providing a scientific basis for further preclinical studies addressing long-term protection and future translational development of an innovative vaccine for human and animal health. Acknowledgements: UFOP, PROPPI, CCA-UFOP, CAPES, INCT-DT, FAPEMIG, CNPq.
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HP - 054 - *Toxoplasma gondii* RH strain inhibits nitric oxide production by differents mechanisms involved iNOS expression depending on the type of peritoneal macrophage of mice providing from distinct microenvironmental conditions

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Toxoplasma gondii is an obligate intracellular parasite capable to infect macrophages. Macrophages control parasite proliferation through nitric oxide (NO) produced by inducible nitric oxide synthase (iNOS) expression. However, virulent strains of *T. gondii* inhibit NO production by iNOS degradation or without affecting iNOS expression depending on the macrophage lineage. It is unknown if *T. gondii* inhibit NO production without degrading iNOS in primary cultures of macrophages. We evaluated if a virulent strain (RH) and a low-virulent strain (ME-49) of *T. gondii* inhibit NO production by modulating iNOS expression in different types of macrophages provinding from distinct microenvironments: resident peritoneal macrophages (ResMO) from health mice, and stimulated peritoneal macrophages (StMO), obtained after 4 days of peritonitis caused by low inoculum of the ME-49 strain. Both types of macrophages, were obtained, seeded and activated with interferon-γ and lipopolysaccharide. After 24h of seeded and activation, ResMO and StMO were infected with *T. gondii* of the RH or ME-49 strains for 2 and 24h. NO was evaluated by the Griess reagent. iNOS expression and development of *T. gondii* were assessed by immunofluorescence and flow cytometer. After 24h of infection with the RH strain, ResMO reduced NO production and percentage of iNOS+ cells. In infected StMO, NO production reduced, but percentage of iNOS+ macrophages did not alter. *T. gondii* growth was higher in ResMO than in StMO. ResMO and StMO Infected with the ME-49 strain did not reduce NO production and the parasite was destroyed in both type of macrophage and, in ResMO, percentage of iNOS+ cells increased while, in StMO, percentage of iNOS+ cells did not change. Therefore, only the virulent RH strain inhibited NO production of ResMO and StMO with reducing percentage of iNOS+cells in ResMO, but not in StMO, demonstrating that pathway of inhibition of NO is different depending on the type of primary cultures macrophages.
Supported by: CNPq, CAPES, FAPERJ, UENF **Keywords:** Toxoplasma gondii;peritoneal macrophages;iNOS.

HP - 055 - Invasion-blocking activity of antibodies to blood-stage *Plasmodium vivax* antigens: *ex vivo* study

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Malaria caused by *Plasmodium vivax* is still the most widely distributed form of the disease outside the African continent. Even so, due to the challenges in maintaining an ex vivo culture for a long period of time, the mechanisms underlying the parasite invasion remain poorly understood. Recently, human monoclonal antibodies against the Apical Membrane Antigen 1 (AMA1) have been shown to block the invasion of reticulocytes in asian *P. vivax* isolates. In this context, our objective is to evaluate the inhibitory effect of anti-PvAMA1 antibodies on reticulocyte invasion using Brazilian Amazonia isolates. Initially, parasites were obtained from Mâncio Lima, Acre, and stored in liquid nitrogen. The cryopreserved isolates were then thawed, washed, and cultured in complete IMDM in a closed system at 37°C. After 36 hours, parasites were treated with 50 nM ML-10 for 6 h and mechanically ruptured to obtain merozoites. For the invasion assay, along with the merozoites, we also added enriched human reticulocytes, the monoclonal antibodies (humAbs 826827 and 824865) and cytochalasin as negative control. After 24 hours of incubation at 37°C, thin smears were analyzed by microscopy and slide digitization to assess invasion. In this preliminary invasion-blocking assay, parasitemia was 0.12%, 0.03%, and 0.00% for the positive control, cytochalasin-treated control, and 826827 (200 µg/mL), respectively, for isolate 262. For isolate 258, parasitemia was 0.08%, 0.04%, and 0.04% for the respective conditions, including 824865 (200 µg/mL). The 826827-treated condition showed reduced parasitemia compared to the positive control, whereas 824865 showed parasitemia comparable to the negative control. Despite the preliminary nature of the assay, these findings support further investigation of the potential inhibitory effects of anti-PvAMA1 antibodies on *P. vivax* invasion. **Supported by:** Fapesp 2026/10284-6
Keywords: Plasmodium vivax;invasion;antibody.

HP - 056 - Evaluation of the action of specific inhibitors of the TcPAQR4 receptor on the physiology and life cycle of Trypanosoma cruzi

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Trypanosoma cruzi is the etiological agent of Chagas disease, a parasitic disease endemic to Latin America and the Caribbean. No vaccine is available, and benznidazole remains the main treatment despite its limited efficacy during the chronic phase. The parasite undergoes a complex life cycle regulated, among other factors, by lipid mediators acting through specific receptors. Platelet-activating factor (PAF), a mammalian lipid mediator, was previously shown by our group to increase metacyclogenesis (epimastigote-to-metacyclic trypomastigote differentiation) and macrophage infection. C18:1 lysophosphatidylcholine (LPC), a structurally related molecule, induces the same effects. We subsequently identified the *T. cruzi* membrane receptor TcPAQR4 as responsible for recognizing both molecules and triggering these phenotypes. We therefore hypothesized that TcPAQR4 inhibitors could represent new therapeutic candidates. A previous in silico study selected 18 candidate inhibitors (MCM1-MCM18). Here, we evaluated their ability to interfere with TcPAQR4-mediated functions. Epimastigotes grown in brain-heart infusion (BHI) medium supplemented with fetal bovine serum were washed in triatomine artificial urine (TAU), incubated for 2 h, and transferred to TAU3AAG medium supplemented with aspartic acid, glutamic acid, L-proline, glucose, and 10 µM of each compound. After 4 days, MCM5, MCM8, MCM16, MCM17, and MCM18 significantly reduced metacyclogenesis compared with the control. After 6 days, MCM5, MCM16, MCM17, and MCM18 remained the most effective. MCM18 maintained approximately 70% of parasites as epimastigotes, whereas the control contained fewer than 10%; the other three compounds yielded 60-70% epimastigotes. MCM13 caused parasite death before differentiation could be evaluated. Future studies will examine the effects of these compounds on RAW 264.7 macrophage infection. We expect the strongest inhibitors of metacyclogenesis to also reduce macrophage infection. Overall, MCM5, MCM16, MCM17, and MCM18 appear to induce alterations in TcPAQR4 physiological responses, supporting their potential as anti-*T. cruzi* compounds, although further studies are required to confirm their efficacy. **Supported by:** **Keywords:** Trypanosoma cruzi;Platelet Activating Factor;Lysophosphatidylcholine.

HP - 057 - Transcriptomic profile of differentially expressed lncRNAs in cerebral malaria in a murine model of Plasmodium berghei ANKA infection

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Cerebral malaria constitutes a lethal form of severe malaria, since blood-brain barrier dysfunction and neuroinflammation, encompassing CD4+ and CD8+ T lymphocytes, microglia and macrophages, remain central to this clinical progression. LncRNAs, a class of transcripts which have more than 200 nucleotides, may exert transcriptional and post-transcriptional regulatory functions upon mRNA levels. This project sought to elucidate the lncRNA-mRNA profile in the murine brain infected by *Plasmodium berghei* ANKA, identifying differentially expressed genes (DEGs). Brain RNA-seq datasets (GSE79702 and GSE162535) were quality-controlled and pseudo-aligned to the murine transcriptome through Salmon v1.10.3. Differential expression analysis was performed using DESeq2 v1.50.2 (|log2FC| > 1 and FDR < 0.05), while cellular composition was estimated by cell type assignment enrichment with CellMarker 2.0. Coexpression modules, identified with CEMiTool v1.32.2, and lncRNA-mRNA pairs within 100 kb were examined for correlated expression across infection days (|r| > 0.7). Such an approach revealed 1,375 mRNAs and 2,582 lncRNAs on day 3 post-infection, 597 mRNAs and 119 lncRNAs on day 5, and 1,244 mRNAs and 535 lncRNAs on day 6, with a core of 432 mRNAs and 65 lncRNAs persisting across all three days. Cell type assignment enrichment of this shared set disclosed a signature dominated by microglia (59.5%), macrophages (12.9%), dendritic cells (6.7%), monocytes (5.5%) and T lymphocytes (4.9%). Among six coexpression modules (M1-M6), M3 stood out for its association with adaptive immune response, encompassing cytotoxic markers of CD4+ (*Cd69, Tnf, Ifng*) and CD8+ T lymphocytes (*Gzmb, Prf1, Havcr2, Pdcd1*). Several M3 genes correlated strongly with neighboring lncRNAs, as in *Gm16548-Sell* and *Gm30934-Cd3g/Cd3e*, suggesting a cis-regulatory mechanism accompanying cerebral malaria progression and positioning these candidates as promising biomarkers for single-cell validation. **Supported by:** FAPESP - 2026/02646-5 **Keywords:** Cerebral Malaria;Transcriptomics;lncRNAs.

HP - 058 - MicroRNAs miR-372 and miR-373 targeting HIF1A determine immunometabolism in macrophages infected with Leishmania infantum

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HIF-1α, hypoxia-inducible factor-1α, constitutes a transcription factor involved in glycolytic gene expression. In macrophages, it is stabilized upon inflammatory stimuli such as LPS, leading to a process of reprogramming carbohydrate and lipid metabolism while inducing inflammation. Given that miR-372/373, ncRNAs that regulate gene expression through seed-mediated pairing with target mRNAs' 3' UTR, were previously found up-regulated in plasma from *Leishmania infantum*-infected patients, this study sought to elucidate their role in HIF1A regulation, integrating target genes and their impact upon the metabolic shift in infected macrophages. THP-1-derived macrophages infected with *L. infantum* were assessed via RT-qPCR, flow cytometry and capillary electrophoresis coupled with mass spectrometry (CE-MS). An integrative in silico analysis was also performed, combining miR-372/373 and HIF1A target prediction, through the multiMiR v1.30.0 and DoRothEA v1.20.0 R packages, with RNA-seq data from these macrophages. We identified 5,476 miR-specific targets, whose differential expression probability was indicaded through logistic regression ($p < 0.05$), 125 HIF1A-specific targets, and their intersection. Infected macrophages showed increased miR-372/373 and decreased HIF1A expression, and a diminished infection rate upon miR-372/373 inhibition. The 61 genes identified as simultaneous targets of both regulators were subjected to KEGG pathway enrichment. During infection in the miR-inhibition compared to the negative control group, glycolysis showed higher enrichment scores than the pentose phosphate pathway. Over-representation analysis of identified metabolites confirmed these same pathways in the metabolomic analysis. These associations support a regulatory link between miR-372/373 and HIF1A, impacting *L. infantum* infection and the glycolytic metabolic shift, a transition possibly indicating an anti-inflammatory state favoring parasite persistence. **Supported by:** FAPESP - 25/10345-2 **Keywords:** Leishmania;MicroRNAs;Trancriptomics.

HP - 059 - CRISPR/Cas9-mediated generation and molecular validation of trypanin mutants in *Leishmania infantum*

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No vaccine against human leishmaniasis is currently available, and existing chemotherapeutic options are limited, expensive, and toxic. The parasite flagellum contributes not only to motility but also to host–parasite interactions, morphogenesis, replication, and pathogenicity, making its components relevant targets for functional studies. Trypanin is a flagellar protein previously characterized in *Trypanosoma brucei* and *T. cruzi*, but its function remains unexplored in *Leishmania*. This study aimed to generate and molecularly validate trypanin mutants in *Leishmania infantum* using CRISPR/Cas9-mediated genome editing as a first step toward investigating the role of this protein in parasite biology. Trypanin mutant lines were generated and screened by PCR, restriction enzyme digestion, and Sanger sequencing, confirming successful genome editing. Growth profiles are currently being characterized, and electron microscopy analyses have been initiated. Preliminary observations suggest that *L. infantum* trypanin mutants exhibit phenotypes similar to those reported in other trypanosomatids, including amorphous cellular masses in culture and impaired motility. Although heterozygous mutants showed growth rates comparable to those of the wild type, the homozygous null mutant displayed reduced growth. These mutant lines provide a foundation for further studies on the effects of trypanin gene disruption on parasite growth, motility, macrophage infection, morphology, and ultrastructure. **Supported by:** CNPQ e FIOCRUZ **Keywords:** Trypanin;Flagellum;Motility.

HP - 060 - Toxoplasma gondii strains selectively regulate H3K4 trimethylation and Polycomb Repressive Complex 2 components in mouse neural cells

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Toxoplasma gondii can invade the developing central nervous system and disrupt neurogenesis. Our previous studies showed that ME49 strain infection impairs proliferation, migration, and neuronal differentiation of mouse cortical neural progenitors. The parasite secretes effectors that reach the host-cell nucleus and modulate transcriptional and chromatin-regulatory pathways. Because T. gondii strains differ in their repertoire and activity of host-directed effectors, we hypothesized that they induce distinct chromatin responses. We investigated H3K4me3, associated with active promoters, and H3K27me3, a repressive modification essential to neural-cell differentiation. Neuro2a cells were infected at MOI 3 with type I (RH), II (ME49), or III (VEG) tachyzoites and analyzed at 12 and 24 hours post-infection by RT-qPCR, immunoblotting, and quantitative immunofluorescence. At 24 hours, VEG increased H3K4me3 abundance, whereas RH reduced expression of the H3K4 methylation-associated factors Setd1a, Rbbp5, and Ash2l. Because H3K27me3 is deposited by Polycomb Repressive Complex 2 (PRC2), we next evaluated its alternative catalytic subunits, EZH1 and EZH2. H3K27me3 remained unchanged, but its regulatory machinery was altered: ME49 reduced Ezh2 expression, whereas VEG decreased total EZH2 protein. Nuclear EZH2 fluorescence increased in RH-parasitized cells but decreased in ME49-parasitized cells. To examine these pathways during congenital infection, pregnant mice received ME49 cysts at embryonic day 10, and fetal cortices were analyzed at E15 and E17. Infection increased H3K4me3 at E15 and Setd1b expression at E17, while Ezh1 and Ezh2 decreased at E17 without changes in H3K27me3. These findings reveal strain- and developmental stage-dependent regulation of H3K4me3 and PRC2 components, suggesting that altered chromatin regulation may contribute to impaired neural development. **Supported by:** VPPCB/Fiocruz, FAPERJ, CNPq. **Keywords:** Toxoplasmosis;Epigenetics; Neurogenesis.

HP - 061 - Regulatory Crosstalk Between of NLRP12 and miR-294 Influences Macrophage Susceptibility to *Leishmania amazonensis* Infection

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Leishmania amazonensis survives and replicates in macrophages by modulating inflammatory pathways and microbicidal mechanisms. NLRP12 regulates innate immunity, whereas miR-294 controls genes linked to macrophage activation. As miR-294 is transcribed from the antisense strand of Nlrp12, these molecules may be functionally connected, but their role remains unclear. This study investigated *Nlrp12* and miR-294 in infected macrophages. Bone marrow-derived macrophages were infected for 4 or 24 hours, and gene expression and infection parameters were assessed by RT-qPCR, optical microscopy, and flow cytometry. miR-294 was inhibited with a specific inhibitor, and *Nlrp12* knockout was performed by CRISPR/Cas9. Public RNA-seq data from C57BL/6 and BALB/c macrophages were analyzed to characterize miR-294 targets and infection-induced transcriptional programs. C/EBPβ and RELA were assessed by siRNA silencing. Infection induced a sustained increase in mature miR-294 and reorganized the transcriptional profile of its predicted targets, particularly in C57BL/6 macrophages. miR-294 inhibition reduced amastigotes per infected macrophage after 4 and 24 hours and altered *Nlrp12*, *Nlrp3*, *Il1b*, and *Il18* expression over time. Conversely, *Nlrp12* knockout increased intracellular parasite burden at both time points and the frequency of infected cells after 24 hours, while altering *pre-miR-294* and inflammasome-related genes. Transcriptomic analyses prioritized *Cebpb* and *Rela* as regulatory factors associated with infection-induced programs. Silencing *Cebpb* or *Rela* reduced the frequency of infected macrophages and parasite-associated fluorescence after 4 and 24 hours. Together, these findings indicate that miR-294 supports intracellular parasite burden, whereas NLRP12 contributes to infection control. C/EBPβ and RELA also favor macrophage susceptibility, supporting a temporal regulatory network that integrates post-transcriptional mechanisms and inflammatory programs during infection.
Supported by: FAPESP (2025/04633-5) **Keywords:** Leishmaniasis;MicroRNA;Macrophages.

HP - 062 - Molecular characterization of *Trypanosoma cruzi* bromodomain factor 2 (*Tc*BDF2).

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Chagas disease, caused by the etiological agent *Trypanosoma cruzi*, is a major neglected tropical disease affecting millions of people worldwide. Gene expression regulation in trypanosomatids is largely polycistronic and occurs primarily through epigenetic and post-transcriptional mechanisms. Bromodomains recognize acetylated histones lysine residues and recruit protein complexes to specific genomic regions. In *T. cruzi*, bromodomain factor 2 (*Tc*BDF2) is essential for its viability, playing a role in cell invasion and differentiation. In this context, this study aims to characterize the biophysical and structural properties of recombinant *Tc*BDF2 and investigate its interaction with the parasite nucleosomes. Recombinant *Tc*BDF2 was expressed in *E. coli* Rosetta (DE3) strain and optimal expression and cell lysis conditions were determined, followed by the development of a purification protocol involving Ni-NTA affinity chromatography and Superdex 75 10/30 PG (Cytiva) size-exclusion chromatography (SEC). The established conditions enabled the production of the protein at high concentrations (3mg/ml) and withan estimated 95% purity. Size-exclusion chromatography indicated that the protein predominantly behaves as a dimeric species with a molecular mass of 27 kDa. Dynamic light scattering (DLS) experiments revealed a consistent homodimeric organization consistent with the SEC results. Comparison across different conditions revealed that the oligomeric state of *Tc*BDF2 may be linked to the recombinant protein concentration and influenced by buffer conditions. The results obtained enabled the establishment of an efficient protocol for *Tc*BDF2 expression and purification. Crystallization assays are currently underway to determine the atomic structure of *Tc*BDF2 as well as histone binding experiments, crucial for subsequent studies regarding chromatin structure regulation.
Supported by: **Keywords:** bromodomain;Trypanosoma cruzi;protein characterization.

HP - 063 - The role of Toxoplasma gondii mitochondrial association factor 1b (MAF1b) in neural progenitor cell biology

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Toxoplasmosis is a widespread disease caused by infection with the obligate intracellular parasite *Toxoplasma gondii*, which secretes effector proteins that manipulate host-cell biology. Among these, MAF1b (Mitochondrial Association Factor 1b) promotes a process named Host Mitochondrial Association, in which host mitochondria closely interact with the parasitophorous vacuole membrane. Since mitochondrial plasticity is important for neural progenitor cell biology, we investigated the role of MAF1b in mitochondrial remodeling and host transcriptional responses. Neuro2a cells were infected with tachyzoites overexpressing MAF1b or the parental type II Prugniaud strain at MOI 3. Previous transcriptomic analyses of infected Neuro2a cells identified alterations in genes related to chromatin regulation and RNA processing across *T. gondii* strains. We tested whether MAF1b would modulate selected components of this response. Neuro2a cells infected with the Pru strain showed increased expression of the chromatin regulator lysine acetyl-transferase 2a (Kat2a), the RNA-processing factor SURP and G-patch domain-containing protein 1 (Sugp1), and of the transcriptional repressor Sin3a compared to uninfected controls, whereas MAF1-infected cultures displayed no such alterations. We also interrogated whether *T. gondii* MAF1b would have a selective effect on mitochondrial quality control. We observed that at 24 hpi, Pru-infected cultures had decreased protein levels of fission marker Fis1, whereas no significant change was observed in MAF1b-infected dishes. Conversely, mitochondrial fission factor (MFF) remained unaltered in Pru-infected cultures, but was significantly decreased in MAF1-b-infected ones. These findings suggest that MAF1b selectively modulate mitochondrial remodeling, as well as host transcriptional responses involving chromatin regulation and RNA processing in neuro progenitor cells, thereby affecting neurogenic programs during in vivo infection. **Supported by:** FAPERJ/CNPq E-26/204.110/2025 (317547)
Keywords: Toxoplasmosis;MAF1b;Mitochondria.

HP - 064 - miR-INFECT - MicroRNAs at the Host–Pathogen Interface: from mechanistic insights to therapeutic potential

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Trypanosomatid infections, including Chagas disease caused by *Trypanosoma cruzi* and leishmaniasis caused by *Leishmania* spp., remain major neglected tropical diseases with a substantial global health burden. Despite their clinical importance, the molecular mechanisms underlying host–parasite interactions remain incompletely understood, limiting the development of effective therapeutic strategies. MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression and have emerged as important modulators of host–pathogen interactions. Although miRNAs have been associated with *T. cruzi* and *Leishmania* infections, their functional roles in trypanosomatid infections remain largely unexplored. Here, we propose to establish a comprehensive functional genomics platform integrating genome-wide miRNA screening, high-content microscopy, and multiparametric image analysis to systematically investigate the role of miRNAs in *T. cruzi* and *Leishmania* infections. Candidate miRNAs will be identified and functionally validated across different host cell types, *T. cruzi* strains, and *Leishmania* species. Their molecular targets and associated cellular pathways will also be investigated to elucidate the mechanisms underlying their effects on parasite infection. In parallel, miRNA expression profiling in clinical samples will provide translational insights and support the identification of potential biomarkers and therapeutic targets. This approach is expected to uncover novel miRNA-mediated regulatory networks involved in trypanosomatid–host interactions and identify molecular pathways that may be exploited for therapeutic intervention. Moreover, the establishment of a functional genomics and high-content screening platform dedicated to parasitic infections will provide a versatile research infrastructure for studying host–pathogen interactions and accelerate the discovery of novel biomarkers and therapeutic strategies for neglected tropical diseases. **Supported by:** FAPESP (2025/15291-8) and Fundação Butantan
Keywords: microRNA screening;functional genomics;Trypanosoma cruzi and Leishmania spp.

HP - 065 - T-Cell Phenotypes Associated with Senescence and Bystander Cytotoxicity in Localized and Diffuse Cutaneous Leishmaniasis

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Localized cutaneous leishmaniasis (LCL) has been linked to cellular senescence, a cellular state that can arise in response to DNA damage. Studies have demonstrated that T lymphocytes within CLL lesions exhibit increased expression of senescence-associeted markers, such as p16, compared with those from healthy individuals. Furthermore, these cells may acquire additional functional properties, including the capacity to mediate bystander cytotoxicity, a TCR-independent mechanism of cell killing involving with the expression of natural killer cell receptors (NKRs) like NKG2D and NKG2C, on T cells. Our current findings suggest that this cellular phenotype is particularly prominent in the highly inflammatory environment of LCL lesions. However, whether this phenomenon is restricted to LCL or also occurs in less inflammatory clinical forms, such as diffuse cutaneous leishmaniasis (DCL), remains unclear. To address this question, we used multiparametric immunofluorescence to evaluate markers of senescence and bystander cytotoxic in CD4+ and CD8+ T cells from LCL and DCL lesions. Phenotypic analyses revealed that, within LCL lesions, both CD4+ and CD8+ T cell populations exhibited an increased frequencies of cells expressing p16 and granzyme B (GZMB). No significant differences were observed in NKG2D or NKG2C expression within either T-cell population. Notably, the combined CD4+NKG2D+GZMB+p16+ and CD8+NKG2D+GZMB+p16+ phenotypes were increased in LCL lesions and appeared to be more prominent in this clinical form. Collectively, these findings reinforce the coexistence of senescence-like and cytotoxic features in T cells from LCL ulcers and suggest that this phenotype may be favored by a highly inflammatory tissue microenvironmental. **Supported by:** CNPq - 445530/2024-3, FAPES - 51677.797.18389.30092022 **Keywords:** American tegumentary leishmaniasis;Cellular senescence;Bystander cytotoxicity.

HP - 066 - CHARACTERIZATION OF SENESCENCE-ASSOCIATED FEATURE IN CD4⁺ AND CD8⁺ T CELLS FROM EARLY AND LATE LOCALIZED CUTANEOUS LEISHMANIASIS LESIONS

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Localized cutaneous leishmaniasis (LCL), mainly caused by *Leishmania braziliensis* in Brazil, progresses from an early papular lesion (<30 days) to a typical ulcerated late lesion. T-cell senescence has emerged as a potential contributor to LCL immunopathology, as senescent T cells display pro-inflammatory and cytotoxic features, and senescence-associated signatures have been identified in both circulating and lesional CD4⁺ and CD8⁺ T cells during *L. braziliensis* infection. However, whether these features are already present in early lesions or become more prominent as the lesional inflammatory microenvironment evolves remains unclear. This study aimed to characterize the expression of the senescence-associated marker p16 in CD4⁺ and CD8⁺ T cells from early and late LCL lesions. Skin lesion samples from eight patients with confirmed *L. braziliensis* infection, comprising four early and four late LCL lesions, were analyzed by multiplex immunofluorescence imaging. CD4, CD8 and p16 were assessed through consecutive rounds of staining and image acquisition using the AxioScan 7, where images were aligned and assembled using in house Python scripts, followed by quantitative image analysis with HALO software. Frequencies of positive cells were compared using the Mann-Whitney test in GraphPad Prism. Late LCL lesions exhibited a significantly higher frequency of p16⁺ cells compared with early lesions. Phenotypic analysis further revealed significantly increased frequencies of both CD4⁺p16⁺ and CD8⁺p16⁺ T cells in late lesions. These findings demonstrate an enrichment of p16-expressing T-cell populations in late LCL lesions, suggesting that senescence-associated features become more prominent as the lesional microenvironment evolves. **Supported by:** FAPES 51677.797.18389.30092022 ; CNPq 445530/2024-3 **Keywords:** Cutaneous leishmaniasis;Immunosenescence;T cells.

HP - 067 - Incorporation of host cholesterol as a mechanism of resistance to azoles in Trypanosoma cruzi

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Cholesterol is the main component of the plasma membrane in most eukaryotes. Some fungi and parasites, such as Trypanosoma cruzi, synthesize ergosterol but incorporate cholesterol from the host. *Candida albicans* and *Nakaseomyces glabratus* uptake cholesterol, which replaces ergosterol when ergosterol biosynthesis is inhibited by azoles, conferring tolerance to this drug. Cholesterol uptake in *T. cruzi* occurs through endocytosis, and the epimastigote stores this cholesterol mainly in structures called reservosomes and in lipid bodies in the cytoplasm; however, it has not been described whether this cholesterol can act as a resistance mechanism of this parasite to azole compounds, since this form of the parasite is quite tolerant to azoles. Therefore, we evaluated whether cholesterol incorporation affects resistance to the ergosterol synthesis pathway. In parallel, we examined an endocytosis-defective mutant that incorporates less cholesterol and has more ergosterol compared to the wild type, analyzing whether it has more or less resistance to azoles. We found that epimastigotes maintained in medium with fetal bovine serum (FBS) presented higher levels of cholesterol than parasites without FBS, but the levels of ergosterol were not affected. However, the parental strains and the mutant growth at 28°C, in the presence or absence of FBS, showed no significant difference in resistance to pozaconazole. In contrast, both were equally susceptible to the drug at 37°C, independently of the presence of FBS, indicating that incorporation of cholesterol cannot replace the ergosterol. Furthermore, fluorescent bodipy-cholesterol is only incorporated in intracellular structures, consistent with the idea that cholesterol is mainly used as a nutritional source for the parasite.
Supported by: FAPESP- Processo 2025/04418-7 **Keywords:** T; cruzi;Cholesterol;Azoles.

HP - 068 - *In vitro* evaluation of chitosan-coated selenium nanoparticles against *Leishmania amazonensis*

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Leishmania amazonensis, belonging to the Trypanosomatidae family, is the etiological agent associated with leishmaniasis, a neglected disease with worldwide distribution and a significant impact on mortality. Available therapeutic options are still limited, being characterized by high toxicity and increasing parasite resistance. In this context, there is a need for new therapeutic compounds, including innovative strategies such as the use of nanoparticles, which have gained relevance in recent research. Selenium nanoparticles (SeNPs) have demonstrated leishmanicidal activity against other Leishmania species, such as *L. major* and *L. tropica*. This study evaluated the efficacy of chitosan-coated selenium nanoparticles (ChSeNPs) against *L. amazonensis in vitro*. ChSeNPs were synthesized using ascorbic acid, sodium selenite, and 0.01% chitosan in 4% acetic acid. ChSeNPs were added to axenic parasite cultures to evaluate growth-inhibitory activity in comparison with a control group. Single-cell ICP-MS analysis was performed to quantify nanoparticle uptake by the parasites, revealing an uptake efficiency of 64%. The obtained half maximal inhibitory concentration (IC_{50}) ranged from 1.9 to 4.9 μg mL^{-1} at 48–96 h of treatment. Transmission electron microscopy revealed a progressive increase in structural damage to the mitochondria and endoplasmic reticulum of the parasite with increasing exposure time and concentration of ChSeNPs. In conclusion, ChSeNPs exhibited promising antiparasitic activity *in vitro*, reinforcing their therapeutic potential and indicating the need for further studies to confirm their efficacy and safety. **Supported by:** apto 203 **Keywords:** Leishmania;nanoparticle;leishmanicidal activity.

HP - 069 - Phylogenetic Inference, Structural Characterisation, and Virtual Screening of a NIMA (Never in Mitosis Gene A)-Related Kinase (NEK) Differentially Expressed Between Trypanosoma cruzi Strains of Distinct Infectivity

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The NIMA-related kinase (NEK) family constitutes a conserved group of serine/threonine protein kinases, associated with cell cycle regulation and cytoskeletal dynamics in eukaryotes. Despite their established relevance in other organisms, NEKs remain poorly explored in *Trypanosoma cruzi*. In the present study, an LC-MS/MS approach was employed to compare the proteomic profile between extracellular amastigote forms of the CL (less infective) and G (more infective) strains. This analysis led to the identification of a serine/threonine protein kinase that exhibited differential expression between the strains, thereby suggesting a potential role in parasite infectivity. Western blot revealed higher protein abundance of this kinase in the CL strain. Conserved domain analysis classified the protein as a member of the NEK family. To ascertain its subfamily, a Maximum Likelihood phylogenetic inference was performed, incorporating *T. cruzi* sequences annotated in TriTrypDB, positioning the protein within the NEK7 clade. The three-dimensional modelling of the catalytic domain was conducted by comparative modelling, followed by structural refinement and validation. A virtual screening against the Brazilian BraCoLi library (comprising 1,176 compounds) identified a lead compound with a binding affinity of -10.19 kcal/mol. This compound was previously synthesised as a trypanothione reductase inhibitor in trypanosomatids. Further analysis of the hotspot mapping revealed the presence of 2 secondary binding regions, which were found to be distinct from the orthosteric ATP-binding site. These findings suggest the existence of allosteric sites that may serve as viable targets for modulators designed to alter kinase conformation, enhancing selectivity relative to human NEKs. These findings underscore the potential of *T. cruzi* NEK7 as a differentially expressed protein that is susceptible to pharmacological inhibition, emphasising its promise for drug repositioning against Chagas disease. **Supported by:** FAPEMIG, CNPq, CAPES **Keywords:** Chagas disease;Molecular docking;Drug repositioning;.

HP - 070 - Metformin treatment reduces lipid accumulation in HepG2 cells infected with Toxoplasma gondii

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Toxoplasma gondii is an obligate intracellular parasite that replicates within parasitophorous vacuoles (PV), recruiting host organelles to obtain nutrients. Unable to synthesize essential lipids, the parasite stimulates the host cell's diacylglycerol acyltransferase, inducing lipid droplet production. Being fat-rich, hepatocytes become preferential targets, potentially triggering hepatic steatosis. Conversely, mitochondrial fusion acts as a cellular defense by optimizing fatty acid oxidation, limiting parasite proliferation. Lipid metabolism regulation strictly depends on AMP-activated protein kinase (AMPK), a metabolic sensor that inactivates Acetyl-CoA Carboxylase (ACC), blocking fatty acid synthesis. Previous studies from our laboratory showed that Plasmodium infection suppresses the AMPK-ACC pathway, generating a lipogenic host profile, whereas AMPK stimulation by metformin contained the infection and attenuated hepatic lipid accumulation. Assuming T. gondii employs analogous metabolic subversion strategies, we hypothesized that metformin treatment preserves AMPK activity, limiting the lipid supply required by the pathogen and favoring cellular defense. Thus, this study aims to analyze lipid profile alterations in hepatocytes during T. gondii infection. Experimentally, HepG2 cell cultures (control and infected with $2x10^6$ parasites) were exposed to 2 mM metformin. Protein content was determined by an adapted Lowry method. Triacylglycerol (TAG) quantification per mg of protein was performed via colorimetric enzymatic assay. Total lipid extraction followed an adapted Bligh and Dyer method. Detailed lipid profiling by Thin-Layer Chromatography (TLC) is currently underway. Preliminary results demonstrated that metformin treatment significantly reduced TAG levels in the infected group compared to the untreated infected group ($p=0.0024$). These data confirm our hypothesis that AMPK activation decreases cellular lipid content, supporting the expectation that lower nutrient availability may restrict energy hijacking by T. gondii and reduce parasite burden. **Supported by:** CNPq e FAPERJ **Keywords:** Toxoplasma Gondim;Hepatocytes;Lipid metabolism.

HP - 071 - Susceptibility profile to antileishmanial drugs and alterations in the miltefosine sensitivity locus in clinical isolates of *Leishmania infantum*.

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Variability in the susceptibility of clinical isolates of *Leishmania infantum* to antileishmanial drugs may influence therapeutic response, reinforcing the importance of monitoring circulating parasites in endemic areas. This study evaluated the susceptibility to trivalent antimony (SbIII), amphotericin B, and miltefosine of 10 clinical isolates of *L. infantum*, obtained from patients with VL in the state of Maranhão, Brazil, and also investigated the relationship between parasite growth profile, susceptibility to miltefosine, and the presence of the miltefosine sensitivity locus (MSL). For this purpose, the *in vitro* growth of five isolates was evaluated by cell counting for seven days. Susceptibility in promastigotes was evaluated by the MTT assay and, in intracellular amastigotes, by determining the infection rate in RAW 264.7 cells. MSL status was analyzed by whole-genome sequencing to detect deletions. IC50 values in promastigotes ranged from 28.14 to 155.2 µM for SbIII, 48.1 to 622.4 nM for amphotericin B, and 7.31 to 25.17 µM for miltefosine. In amastigotes, the EC50 for miltefosine ranged from 1.56 to 7.14 µM. Of the 10 isolates, four showed complete deletion and one showed partial deletion of the MSL. Complete or partial deletion was associated with greater tolerance to miltefosine, with significantly higher EC50 values in these parasites than in MSL+ parasites ($p < 0.0001$). Among the isolates evaluated for growth, those with complete or partial deletion showed a higher growth rate and higher EC50 values in amastigotes, suggesting an association between MSL alterations, growth rate, and reduced susceptibility to miltefosine. The results demonstrate the phenotypic heterogeneity of the isolates regarding antileishmanial drugs and confirm the circulation, in Maranhão, of isolates with alterations in the MSL, reinforcing the importance of monitoring phenotypic and genetic markers for resistance surveillance.
Supported by: Rede FitoAmazônia. CNPq/MCTI/FNDCT Nº 19/2024.; PPSUS. FAPEMA/CNPq/Decit-SECTICS-MS/SES-MA nº 02/2025.; CAPES – Código de Financiamento 001. **Keywords:** Visceral leishmaniasis;drug susceptibility;MSL locus.

HP - 072 - Hepatic Lipid Reprogramming in Leishmaniasis: The Role of the AMPK/ACC Axis

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INTRODUCTION: *Leishmania* relies on modulating host lipid metabolism to survive. Chronic *L. amazonensis* infection causes systemic metabolic reprogramming, but the underlying signaling mechanisms and early pharmacological interventions require elucidation. **OBJECTIVE:** To investigate how *L. amazonensis* alters hepatic lipid metabolism and if AMPK activation by metformin mitigates these alterations during acute and chronic phases in female C57BL/6 mice. **METHODS:** Eight-week-old female C57BL/6 mice were infected with *L. amazonensis* to evaluate the hepatic lipid profile during chronic pathogenesis (225 dpi). Lipid profiles and lipogenic pathways were analyzed using TLC, colorimetric assays, and Western blotting. A subsequent stage evaluated metformin modulation (150 mg/kg, 14-day pre-treatment) across acute (45 dpi) and chronic (225 dpi) stages. **RESULTS:** Chronically infected mice exhibited increased hepatic TAG ($p=0.004$; TLC $p=0.02$), 1,2-diacylglycerol, free cholesterol, and free fatty acids ($p=0.02$ each). Monoacylglycerols and oxysterols decreased ($p=0.02$). Phospholipids shifted, showing elevated phosphatidic acid, phosphatidylethanolamine ($p=0.02$ each), phosphatidylinositol, and sphingomyelin ($p=0.004$ each). This pro-lipogenic state correlated with reduced inhibitory phosphorylation of AMPK and its target, ACC ($p=0.04$ each), confirming *de novo* lipogenesis activation. Acutely, metformin significantly reduced lesion size and parasite burden ($p=0.0001$), with TLC indicating a tendency to normalize TAG and overall lipid profiles. **CONCLUSION:** *L. amazonensis* induces hepatic lipid reprogramming by suppressing host AMPK signaling, thereby activating ACC to drive lipogenesis for long-term persistence. Early pharmacological AMPK activation by metformin reduces parasite load and restores lipid homeostasis, identifying the AMPK/ACC pathway as a viable therapeutic target.
Supported by: CAPES **Keywords:** Leishmania amazonensis;Lipid Metabolism;Host-parasite interaction.

HP - 073 - Modelling next-generation human colonic organoids to investigate neuro-immune crosstalk during enteric parasitic infections

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Objective: To investigate how the enteric nervous system (ENS) and innate immune system coordinate responses to parasitic infection using a human-relevant model.
Methods: We developed next-generation human colonic organoids (HCOs) incorporating both ENS elements and innate immune cells. These complex HCOs were infected with *Trypanosoma cruzi*, the protozoan parasite responsible for digestive Chagas disease. We assessed infection dynamics, host responses, and neuro-immune signalling using immunofluorescence, live imaging and transcriptomic analysis.
Results: *T. cruzi* successfully established infection in ENS-immune HCOs, leading to structural disruption, altered cellular integrity, and an inflammatory profile. Comparative analyses with control organoids lacking ENS or immune components indicated the critical role of neuro-immune crosstalk in modulating infection outcomes.
Conclusion: Our model demonstrates that the ENS and innate immune system jointly shape the intestinal response to *T. cruzi*. This human-relevant system provides a powerful platform to dissect host-pathogen interactions and may guide future host-directed therapeutic strategies for enteric parasitic diseases.
Supported by: Horizon Europe - Marie Skłodowska-Curie Actions; ANR **Keywords:** T; cruzi;Neuro-immune cross-talk;Gut organoids.

HP - 074 - Circulating γδ T Cells Display an Increased CCR5$^+$ Phenotype in Localized Cutaneous Leishmaniasis

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Localized cutaneous leishmaniasis (LCL) is characterized by ulcerative lesions in which an exacerbated immune response contributes to persistent inflammation and tissue damage. Cytotoxic CD8$^+$ T cells are well-established mediators of LCL immunopathogenesis, and CCR5-dependent T-cell recruitment contributes to their accumulation within leishmanial lesions. Beyond conventional αβ T cells, recent single-cell transcriptomic analyses have revealed an enrichment of γδ T cells in CL skin, highlighting this population as a potentially component of the lesional immune response. However, the mechanisms governing their recruitment and the functional profile of γδ T cells during human LCL remain poorly understood. We therefore investigated the phenotypic, cytotoxic, and migratory profiles of circulating γδ T cells in patients with LCL. Peripheral blood mononuclear cells (PBMCs) from LCL patients and healthy controls were analyzed by multiparametric flow cytometry. γδ T-cell differentiation and cytotoxic potential were assessed based on the expression of CD57, granzyme B, CD107a, NKG2A, NKG2C, and NKG2D, while CCR5 was evaluated as a marker related to tissue-homing potential. No significant differences were observed in the differentiation or cytotoxic profiles of circulating γδ T-cell between LCL patients and healthy controls. In contrast, LCL patients exhibited a higher frequency of γδ$^+$CCR5$^+$ T cells. Together, these findings suggest that γδ T-cell alterations in the circulation are preferentially linked to migratory rather than cytotoxic features. Given the reported enrichment of γδ T cells within CL lesions, increased CCR5 expression may facilitate their recruitment to inflamed skin, where their functional contribution to the local immune response remains to be determined. **Supported by:** CNPQ - 445530/2024-3 **Keywords:** localized cutaneous leishmaniasis;γδ T lymphocyte;CCR5.

HP - 075 - HALOGENATED NAPHTHOQUINONES AS POTENT AGENTS AGAINST *Trypanosoma cruzi*: STRUCTURE-ACTIVITY RELATIONSHIP AND MITOCHONDRIAL DYSFUNCTION

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According to the World Health Organization, neglected tropical diseases mainly affect poor populations in more vulnerable regions around the world, Chagas disease being one of them. The available chemotherapy is limited and presents several problems such as high toxicity, reinforcing the need to search for new alternatives for treatment. To minimize possible sideeffects, new strategies have been developed based on biological processes established during the parasite's relationship with its hosts. It is well-described in the literature that reactive oxygen and nitrogen species are important mechanisms for combating *T. cruzi* in the vertebrate host. In this scenario, quinones are characterized by their oxidation-reduction capacity, leading to the production of reactive oxygen species, known to cause damage to biological membranes and interfering with different biosynthetic pathways. Previous data o four group demonstrated that the parasite mitochondrion was affected by naphthoquinones through a blockage of the activity of the I-III complexes, impairing electron transport flux and producing mitochondrial reactive oxygen species. Here, we performed an in-depth characterization of six halogenated naphthoquinones previously identified as highly active against bloodstream trypomastigotes, investigating the relationship between their chemical structure, trypanocidal activity, and mitochondrial effects. All compounds exhibited potent activity on the three *T. cruzi* developmental stages, with IC_{50}/24 h values in the low micromolar to nanomolar range, showing the highest efficacy against intracellular amastigotes. Moreover, all compounds displayed promissing selectivity índices; HaN#2 and HaN#4 exhibited particularly high selectivity indices (513.3 and 258.6, respectively). Ultrastructural analysis revealed pronounced mitochondrial swelling in epimastigotes treated with HaN#2, HaN#4, and HaN#5, consistent with mitochondrial membrane potential collapse and increased reactive oxygen species production. Furthermore, pretreatment with the mitochondria-targeted antioxidant MitoTEMPO markedly reduced the trypanocidal activity of HaN#2, suggesting that mitochondrial ROS generation contributes to its mechanism of action. Taken together, our findings demonstrate that mitochondrial dysfunction is a major target of halogenated naphthoquinones and reveal a correlation between trypanocidal activity and mitochondrial oxidative damage. **Supported by:** FAPERJ **Keywords:** Trypanosoma cruzi;Chemotherapy;Quinones.

HP - 076 - Macrophage Lipid Metabolism is Redirected Towards Endogenous Lipogenesis and PUFA Biosynthesis by *Leishmania amazonensis*

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Leishmaniases are neglected tropical diseases caused by *Leishmania*, whose successful infection depends on macrophage subversion. Lacking complete de novo lipid biosynthesis, these parasites act as auxotrophs, acquiring essential molecules from host cells to build membranes and sustain energetic demands. We hypothesize infection promotes endogenous lipid biosynthesis in macrophages, causing pronounced lipid storage utilized by the parasite. This work characterizes lipid content alterations in human macrophages induced by *L. amazonensis* infection. THP-1 macrophages were infected with *L. amazonensis* Josefa (10:1) and analyzed up to 48h. Lipid profiles and storage were evaluated using HPTLC, GC-MS, and confocal microscopy with BODIPY staining. To trace lipid origins, radiolabeled assays used [³H]LDL, [³H]HDL, and [³H]palmitate. HPTLC revealed downregulation of key structural phospholipids (phosphatidylcholine, phosphatidic acid, phosphatidylserine, phosphatidylinositol), and a marked upregulation of cholesteryl esters and diacylglycerols at 24h. This metabolic rerouting preceded extensive neutral lipid accumulation at 48h, characterized by storage of triacylglycerols, cholesteryl esters, and free fatty acids. This corresponded to increased lipid droplets, dynamic centers for lipid storage and inflammatory mediator biosynthesis, confirmed by BODIPY. Radiolabeled assays indicated that while [³H]palmitate uptake remained unchanged, internalization of exogenous lipoproteins ([³H]LDL, [³H]HDL) was suppressed. This indicates lipid accumulation is driven by hyperactivation of endogenous synthesis rather than extracellular scavenging. Furthermore, the infection extensively altered polyunsaturated fatty acid (PUFA) profiles, specifically promoting the accumulation of omega-6 pathway intermediates. The simultaneous detection of elevated dihomo-gamma-linolenic acid (DGLA, C20:3), arachidonic acid (AA, C20:4), and osbond acid (C22:5) reveals an active elongation and desaturation cascade sustained by the infected host cell, alongside exclusive infection markers like 10-heptadecenoic acid (C17:1). This cascade provides the lipid pool for immunomodulatory eicosanoids. Overall, *L. amazonensis* establishes an endogenous lipogenic shunt, creating a lipid-rich intracellular niche essential for survival and proliferation. **Supported by:** CNPQ; FAPERJ. **Keywords:** Leishmania amazonensis;Lipid metabolism;Polyunsaturated fatty acids.

HP - 077 - Characterization of lncRNA and mRNA expression in Leishmania major infected macrophages using integrative bioinformatics

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Macrophages constitute the main intracellular niche for *Leishmania major* and undergo extensive transcriptional reprogramming during infection, affecting inflammatory signaling, metabolism, and parasite control. Long non-coding RNAs (lncRNAs) regulate gene expression at transcriptional and post-transcriptional levels and may participate in these responses, although their role in *L. major*-infected macrophages remains poorly characterized. Therefore, this study aimed to characterize lncRNA and mRNA expression in murine macrophages during infection, identify differentially expressed transcripts and coexpression modules, and investigate lncRNA-mRNA associations linked to host-response processes. Candidate pairs will subsequently be evaluated in an independent dataset to assess their transcriptional consistency over time. Public bulk RNA-seq data from murine peritoneal macrophages were analyzed using an integrative bioinformatics approach. In GSE189385, differential expression was assessed with DESeq2 and coexpression modules were identified with CEMiTool. We identified 4,541 differentially expressed mRNAs, including 2,224 upregulated and 2,317 downregulated genes, and 1,630 differentially expressed lncRNAs, comprising 773 upregulated and 857 downregulated transcripts.
Three coexpression modules contained DEGs: M1 comprised 250 DEGs, including 189 lncRNAs and 61 mRNAs; M2 comprised 907 DEGs, including 189 lncRNAs and 718 mRNAs; and M3 comprised 721 DEGs, including 381 lncRNAs and 340 mRNAs. Functional enrichment of differentially expressed mRNAs with enrichR using Gene Ontology Biological Process (GO-BP) revealed distinct profiles. M1 was associated with stress response and cell differentiation, M2 with RNA metabolism, RNA polymerase II transcription, and M3 with vesicle-mediated transport. Spearman correlation identified lncRNA-mRNA pairs linking lncRNAs to mRNAs involved in enriched processes. In M2, *Tnf* (padj = 2.55×10^{-122}; log2FC = 5.44) was present in all enriched GO-BP terms analyzed. Gm44140 (padj = 1.81×10^{-15}; log2FC = 4.14), Gm32287 (padj = 1.87×10^{-10}; log2FC = 2.60), and Gm72923 (padj = 6.09×10^{-9}; log2FC = 3.79) were selected as the top three *Tnf*-correlated lncRNAs based on Spearman's ρ and adjusted p-value. Candidate pairs will be evaluated in PMC4696162 using a DESeq2 likelihood ratio test to characterize infection-associated expression dynamics over time and assess transcriptional consistency. **Supported by:** FAPESP: 2026/02660-8 **Keywords:** Leishmania major;Transcriptomics;Long non-coding RNA.

HP - 078 - Following Every Parasite: DNA Barcoding as a Tool to Track *Trypanosoma cruzi* Sub-clonal Population Dynamics

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Trypanosoma cruzi undertakes repeated cycles of host cell invasion, multiplication, egress and re-invasion, gradually shaping Chagas disease pathology. Migration within the host, involving both local tissue residence and traversal across endothelial barriers to the bloodstream adds further complexity. Symptoms and response to drug treatments are highly variable amongst infected individuals, even in apparently clonal experimental infection models. Thus, unexplored heterogeneity within parasite populations likely contributes to within-host infection dynamics, disease progression and treatment outcomes. Here, we have adopted a Bar-seq approach enabling us to track sub-clonal parasite lineages in space and time. We generated bioluminescent-fluorescent, DNA-barcoded epimastigote populations of two *T. cruzi* cell lines, TcVI-CLBR and TcI-JR with up to 500 unique subclones per library that can be quantified by an optimised amplicon sequencing pipeline. As proof-of-concept, we investigated parasite population dynamics during differentiation to infectious metacyclic forms and host-cell invasion. Our data demonstrate the feasibility of combining high-complexity DNA barcoding with next-generation sequencing to trace the impact of key life-cycle bottlenecks on *T. cruzi* sub-clonal population structure.
We are currently extending this approach to investigate parasite responses to anti-parasitic drug pressure, with a particular focus on the emergence and selection of benznidazole-resistant populations. By enabling the tracking of individual parasite lineages over time, this platform provides a powerful tool for identifying fitness determinants associated with differentiation, invasion, dissemination, and drug resistance. Ultimately, these studies will contribute to a deeper understanding of the biological processes underlying *T. cruzi* infection. **Supported by:** **Keywords:** Chagas disease;Trypanosoma cruzi;Pathogenesis.

HP - 079 - Characterization of the Antiparasitic Activity of Trypanosoma cruzi by High-Content Screening: Insights into Mechanism-of-Action Studies

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Chagas disease, caused by the protozoan Trypanosoma cruzi, remains an important public health problem, highlighting the need for new drug candidates and strategies to characterize their effects on the parasite. High-Content Screening (HCS), based on automated microscopy, enables the assessment of compound activity against intracellular forms, providing parameters such as IC_{50}, selectivity index, and maximum activity, while also allowing temporal and proliferation dynamics analyses. In this study, we evaluated the antiparasitic activity and kinetics of action of benznidazole, nifurtimox, and posaconazole against the Sylvio X10/1 and YH10-S clones of T. cruzi. U2OS cells were infected with trypomastigotes and exposed to the compounds, with activity assessed by HCS from 0 to 144 hours. Parasite proliferation dynamics and doubling time were analyzed in U2OS and LLC-MK2 cells between 24 and 96 hours, aiming to characterize the parasite–host interaction and investigate the behavior of metabolically active and quiescent subpopulations (“persisters”). For Sylvio X10/1, benznidazole showed an IC_{50} of 0.1 µM, SI >4332.5, and maximum activity of 106%; nifurtimox showed an IC_{50} of 2 µM, SI = 29, and maximum activity of 96%; and posaconazole showed an IC_{50} <0 µM, SI >111, and maximum activity of 54%. For YH10-S, the IC_{50} values were 2.5, 0.4, and 0.002 µM, respectively, with SI >158, >260, and >560. Benznidazole and nifurtimox showed a progressive increase in activity, whereas posaconazole exhibited slower activity. Replication dynamics varied between strains and host cells, with different doubling times. As a perspective, integration of HCS with Cell Painting will expand the characterization of compound-induced phenotypic alterations. Novel compounds will serve as case studies to assess phenotypic profiles and generate hypotheses on their yet-unexplored mechanisms of action, compared with compounds of known MoAs. **Supported by:** FAPESP 2026/16738-9 **Keywords:** Trypanosoma cruzi;;High-Content Screening;Cell Painting;.

HP - 080 - Evaluation of the Impact of PAF Receptor Inhibitors on *Trypanosoma cruzi* Physiology

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Chagas disease, first described by Carlos Chagas, is caused by the protozoan *Trypanosoma cruzi* and is transmitted primarily by triatomine insect vectors. However, transmission may also occur through oral contamination, blood transfusion, organ transplantation, and congenital infection. Endemic to Latin America, the disease comprises acute and chronic phases, lacks an available vaccine, and its treatment relies mainly on benznidazole and nifurtimox, both of which present important therapeutic limitations, particularly during the chronic stage of the disease. During the infectious process, the lipid mediators platelet-activating factor (PAF) and lysophosphatidylcholine (LPC), particularly LPC C18:1, play key roles in the modulation of the immune response, metacyclogenesis, and macrophage infection. In this context, the present study aimed to evaluate the potential of specific inhibitors targeting the *T. cruzi* PAF receptor, TcPAQR4, with the goal of identifying novel therapeutic strategies capable of interfering with the parasite's life cycle and pathogenicity. A previous study conducted by our group preselected eighteen candidate molecules as potential TcPAQR4 inhibitors through molecular docking analysis. In the present study, these compounds were initially screened *in vitro* at a concentration of 10 µM. The results demonstrated that compounds MCM 2 and MCM 13 were capable of inhibiting epimastigote growth. These findings were further confirmed by fluorometric assays using Alamar Blue. Toxicity assessment was performed using the GUSAR platform through *in silico* prediction of the median lethal dose (LD_{50}) across four potential routes of administration, indicating slightly higher predicted toxicity than benznidazole. Conversely, fluorometric Alamar Blue assays performed with murine RAW 264.7 macrophages demonstrated that the MCM compounds did not exert significant cytotoxic effects on host cells. As a future perspective, the safety of these compounds will be further investigated through survival curve assays using the nematode *Caenorhabditis elegans*, a widely employed model for *in vivo* toxicity studies. Taken together, these preliminary findings suggest that TcPAQR4 represents a promising therapeutic target for the development of novel anti-*T. cruzi* agents. The identification of specific inhibitors of this receptor may contribute to expanding the currently available therapeutic options for Chagas disease. **Supported by:** **Keywords:** Trypanosoma cruzi;Platelet Activating Factor;Lysophosphatidylcholine.

HP - 081 - NK-like activity throughout NKG2D in CD4 + and CD8 + as a Potential Mechanism of Tissue Damage in LCL.

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Localized Cutaneous Leishmaniasis (LCL) is a parasitic disease caused by protozoa of the Leishmania genus, characterized by ulcerated skin lesions associated with chronic inflammatory immune response. Persistent antigenic stimulation and continuous lymphocyte proliferation during infection may induce immunosenescence in T cells, leading to the acquisition of natural killer cell receptors (NKRs), such as NKG2D. Aditionally, i nflammatory environments are known to promote cellular stress conditions and upregulate the NKG2D ligand, MICA/MICB. Taken together this scenario could contribute to the development of non-specific cytotoxic phenotype along with MICA/MICB expression in cells resulting in a mechanism of non-specific killing. Using multiplex immunofluorescence, this study aimed to characterise the expression of NKG2D in $CD4^+$ and $CD8^+$ cell populations within lesions from patients with LCL compared with healthy controls (HC), as well as to investigate the spatial distribution of these cells and their proximity to potential target cell populations, including $Vimentin^+$ and $CD68^+$ cells. Our results demonstrated an increased abundance of $CD4^+$ and $CD8^+$ cells expressing NKG2D within LCL lesions, indicating the accumulation of this distinct phenotype at the site of infection. Furthermore, spatial analysis revealed the presence of $CD68^+$ and $Vimentin^+$ cells within a 10 µm distance of $CD4^+GZMB^+NKG2D^+$ and $CD8^+GZMB^+NKG2D^+$ cells, with a greater average number of neighbouring cells observed around the $CD8^+$ phenotype. Taken together, these findings suggest the presence of an NK-like cytotoxic T cell population that could contribute to the extensive tissue damage by promoting the killing of immune and stromal cells, a mechanism that can be potentially associated with ulcer development and pathology in patients with LCL. **Supported by:** Financiadora e Código do processo FAPES 51677.797.18389.30092022 , CNPq: 445530/2024-3 **Keywords:** Immunopathology ;Cytotoxicity;Localized Cutaneous Leishmaniasis.

HP - 082 - Molecular detection of Leishmania parasites in a human population from a rural settlement in a region of the Amazon Rainforest

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Leishmaniasis, caused by the genus *Leishmania*, is an anthropozoonotic disease characterized by the interaction of parasites, invertebrate vectors, and vertebrate hosts. The city of Cruzeiro do Sul, Acre, located in the Amazon Rainforest biome, is an endemic region for several *Leishmania* species and other similar tropical pathogens. Considering the high biodiversity of hosts and vectors, and the social vulnerability of the human populations in this region, a reliable and precise detection of *Leishmania* parasites directly from biological samples deems necessary. This study aims to investigate the molecular presence of *Leishmania* parasites in a human population from a rural settlement in Cruzeiro do Sul - AC. Human blood, plasma and saliva samples from 154 patients were collected in 2024, the DNA extracted and submitted to molecular identification of *Leishmania* by PCR and qPCR amplification of the chitinase encoding gene and a PCR amplification of the first portion of the V7V8 region of the 18S SSU rRNA (18S 01-687). The positive results were sequenced by Sanger's method and submitted to BLAST analysis. The qPCR of the chitinase amplified 04 fragments from blood samples, 02 from plasma and 03 from saliva, while the PCR tests for the same molecular marker were all negative. The PCR of the 18S 01-687 amplified 06 fragments from blood samples, 10 from plasma and 12 from saliva. After sequencing and BLAST analysis, 07 fragments were obtained which showed homology with *Leishmania* sequences from the Genebank, being 03 of them for the chitinase in saliva samples and the other 04 for the 18S 01-687, being two of them saliva samples, one for blood and the other for plasma. These results showed that there is a likely *Leishmania* presence in the human population of this area, and it also demonstrated the value of these molecular markers for the detection of *Leishmania* parasites directly from biological samples, notably from saliva, which is a low-cost and non-invasive method for sample collection. **Supported by:** FAPESP: 21/06669-6, 22/10392-2; CAPES: 88887.721976/2022-00 **Keywords:** Leishmania;molecular diagnosis;amazon rainforest.

HP - 083 - Leishmania amazonensis promastigotes induce the release of DNA extracellular traps (DETs) in human macrophages via TLR2, TLR4, and NLRP3 inflammasome activation.

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Leishmaniases are diseases caused by protozoan parasites of the genus *Leishmania*, transmitted by hematophagous vectors of the Phlebotominae subfamily. The disease presents two main clinical forms: tegumentary and visceral leishmaniasis, the latter being fatal if left untreated. In Brazil, *Leishmania amazonensis* is a highly relevant species, primarily associated with the tegumentary form. Macrophages, the target cells for *Leishmania*, possess several microbicidal mechanisms, including phagocytosis and the extrusion of macrophage extracellular traps (METs/DETs). Similar to neutrophil extracellular traps (NETs), DETs are composed of a DNA backbone associated with various proteins and exhibit microbicidal activity. Although *Leishmania* infects macrophages, there is a lack of information regarding its ability to induce DETs in these cells. Here, we demonstrate that *Leishmania amazonensis* promastigotes induce DET release in human macrophages. Fluorescence microscopy confirmed that these traps contain citrullinated histone H3 (H3cit), myeloperoxidase (MPO), and elastase, displaying a morphology similar to NETs. This release depends on the interaction between promastigotes and TLR2 and TLR4 receptors, as pharmacological inhibition of these receptors reduced DET release. Furthermore, inhibitors of peptidylarginine deiminase (PAD4), calcium, and the NLRP3 inflammasome significantly reduced promastigote-induced DET release by more than 61%. We also show, using the resazurin assay, that DETs exert direct toxicity against promastigotes. The infection index was significantly reduced in macrophages treated with purified DETs, and DNase treatment abolished this inhibitory effect. Taken together, our results demonstrate that DETs interfere with parasite-macrophage interaction and that the cleavage of the DNA backbone reverses the leishmanicidal effect of these traps. **Supported by:** CAPES, FAPERJ E CNPQ **Keywords:** Leishmania ;Macrophages ;DNA Extracellular Traps (DETs).

HP - 084 - Identification and CRISPR/Cas9 deletion of potential molecular targets implicated in *Leishmania infantum* macrophage infection

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Considered a public health problem in Brazil, leishmaniases are parasitic diseases caused by protozoa of the genus *Leishmania*, whose infective form for the mammalian host is the metacyclic promastigote. Current therapeutic options are associated with high toxicity and inconvenient routes of administration, highlighting the identification of new targets that interfere with the parasite life cycle and reduce macrophage infection as a promising strategy for the development of safer, more specific, and more effective drugs. In this context, Li1, a cyclic and biotinylated 12-amino-acid peptide obtained from a phage display library, interacts with the surface of *Leishmania infantum* and reduces amastigote viability in murine macrophages by 43.80% and 31.25% when pre-incubated with promastigotes and macrophages, respectively. However, this reduction is likely associated with interference in promastigote–macrophage interaction, since Li1 exhibits neither anti-promastigote nor anti-amastigote activity and shows no cytotoxicity (IC50/CC50 > 1.0 mg/mL). Therefore, Li1 was employed as a bait molecule to identify novel molecular targets through a pull-down assay using a streptavidin matrix and total protein extract from *L. infantum*. Mass spectrometry analysis of the eluate, combined with a search of the UniProt database, identified four proteins that interact with the peptide. To assess the essentiality of these proteins for parasite internalization, *L. infantum* knockout strains are being generated using CRISPR/Cas9 by deleting the genes encoding the identified proteins, followed by selection with puromycin. Two strains with complete knockout of both alleles have already been successfully obtained, enabling subsequent functional validation assays to investigate their involvement in parasite internalization and infection. **Supported by:** Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) nº 2025/22164-2, Financiadora de Estudos e Projetos (FINEP) n°01.23.0034.00 (0419/22). **Keywords:** Host–parasite interaction;CRISPR/Cas9;Leishmaniasis.

HP - 085 - In Vitro Antileishmanial Activity of Ruthenium Complexes against *Leishmania infantum*

DA SILVA, F.O.; NUNES, S.M.S.; BARCELOS, A.C.A.; OSTOLIN, T.L.V.D.P.; CELESTINO, R.M.C.; ALBUQUERQUE, M.C.V.T.; SANTOS, L.I.D.C.R.E.; VIEIRA, P.M.D.A.; CORRÊA, R.D.S.; REIS, A.B.; SOARES, R.D.D.O.A.; ROATT, B.M..
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Brazil accounts for the majority of visceral leishmaniasis (VL) cases in the Americas, caused by *Leishmania infantum*, reinforcing the need for new therapeutic alternatives. In this study, we evaluated four ruthenium complexes (C1-8HQ, C2-SO_3HQ, C3-ClHQ, and C4-NO_2HQ) against different forms of *L. infantum*. The complexes were characterized by UV-Vis spectroscopy, NMR, and mass spectrometry, and subjected to *in silico* analyses using ADMETlab 2.0. Cytotoxicity was determined using the sulforhodamine B (SRB) assay, while leishmanicidal activity was evaluated by the MTT assay. Infection rate and parasite burden were determined by flow cytometry, and cytokine production was evaluated in infected macrophages. C1-8HQ, C2-SO_3HQ, and C3-ClHQ exhibited CC_{50} values >50 µmol L^{-1}, whereas C4-NO_2HQ showed greater cytotoxicity, with CC_{50} values of 39.16 and 24.38 µmol L^{-1} after 24 and 48 h, respectively. All complexes exhibited IC_{50} values >50 µmol L^{-1} against promastigotes. However, against intracellular amastigotes, C1-8HQ showed IC_{50} values of 27.26 and 26.80 µmol L^{-1}, whereas C3-ClHQ showed greater activity, with IC_{50} values of 22.02 and 14.67 µmol L^{-1} after 24 and 48 h, respectively. C3-ClHQ produced the greatest reductions in intracellular parasite burden, reaching 93% and 86% after 24 and 48 h, respectively. In contrast, C4-NO_2HQ reduced parasite burden by 45% at both time points. Treatment with C1-8HQ and C3-ClHQ modulated the inflammatory profile of macrophages, reducing MCP-1 and TNF levels. Overall, C1-8HQ and, particularly, C3-ClHQ exhibited the most promising profiles, standing out for their activity against intracellular amastigotes and the substantial reduction in parasite burden. These results indicate C3-ClHQ as a promising candidate for further studies aimed at elucidating its mechanisms of action and evaluating its antileishmanial potential. Acknowledgements: UFOP, PROPPI, CCA-UFOP, CAPES, INCT-DT, FAPEMIG, CNPq. **Supported by:** CAPES - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (001). FAPEMIG - Fundação de Amparo à Pesquisa do Estado de Minas Gerais (APQ-02494-22; APQ-02961-24; APQ-02835-25). CNP-q - Conselho Nacional de Desenvolvimento Científico e Tecnológico - MCTI/CNPq/CTBIOTEC. INCT-DT - Instituto Nacional de Ciência e Tecnologia em Doenças Tropicais **Keywords:** Ruthenium Compounds;Immunomodulation;Cytotoxicity Tests.

HP - 086 - FeBS metallocomplex effects on malaria-associated pathology and nitric oxide production by RAW 264.7 macrophages

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Malaria remains a major global health problem, and the emergence of parasite resistance reinforces the need to investigate new therapeutic compounds and their interactions with host responses. Previous *in vivo* studies demonstrated that the FeBS metallocomplex [Fe(HL1)Cl_3] modifies the progression of *Plasmodium chabaudi* infection in *BALB/c* mice, reducing parasitemia progression, delaying clinical manifestations, prolonging survival and preserving hepatic and splenic architecture. These findings raised the question of whether FeBS may also influence host cellular responses associated with infection. Therefore, the present study investigated the effect of FeBS on nitric oxide production by RAW 264.7 macrophages. Activated (M1) and non-activated (M0) macrophages were exposed to different concentrations of FeBS (12.5, 25, 50, 125 and 1250 µM), and nitrite (NO_2^-) production was evaluated as an indirect indicator of nitric oxide production. As expected, activated macrophages exhibited markedly higher NO_2^- levels than non-activated cells. Exposure to FeBS did not produce a pronounced increase in NO_2^- levels in either macrophage condition across the concentrations evaluated. In activated macrophages, NO_2^- production remained relatively high after FeBS exposure, with an apparent reduction at the highest concentration, whereas non-activated macrophages maintained low NO_2^- levels throughout the treatments. Together with the previously observed *in vivo* effects, these preliminary findings suggest that the biological activity of FeBS during experimental malaria may not depend on a pronounced induction of macrophage nitric oxide production under the conditions evaluated. Further studies investigating additional cellular and molecular pathways are required to clarify how FeBS influences parasite–host interactions and to better characterize the mechanisms underlying its potential antimalarial activity. **Supported by:** FAPERJ: E26/201.689/2026 **Keywords:** Plasmodium chabaudi;Nitric oxide ;host response.

HP - 087 - Impact of Trypanosoma rangeli Infection on Lipid Transport During Oogenesis in Rhodnius prolixus.

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Trypanosoma rangeli is a protozoan of the family Trypanosomatidae found in the salivary glands of Rhodnius prolixus. During its life cycle, the triatomine bug acquires trypomastigote forms when feeding on an infected host. Epimastigote forms emerge in the foregut; they interact with the intestinal wall, invade the hemolymph, and penetrate hemocytes, where they differentiate. New forms are released upon the rupture of these cells. Lacking complete lipid metabolism, the parasites must acquire lipids from the invertebrate host to survive and proliferate. Infection can cause various complications for the insect, including death. Thus, T. rangeli could act as a potential biological control agent against Chagas disease by reducing triatomine populations. This study aims to evaluate the impact of T. rangeli infection on lipid transport during R. prolixus oogenesis. To this end, 10^4 parasite forms were injected 24 hours after a blood meal. Three days later, the insects were dissected, and the ovaries were analyzed using thin-layer chromatography. In a separate experiment, tritiated palmitate was injected to investigate the infection's influence on lipid dynamics. Assays such as electrophoresis were used to assess lipophorin and vitellogenin levels in the hemolymph. Results showed alterations in the lipid levels of infected insects, with a reduction in triacylglycerol (TAG), fatty acids (FA), and diacylglycerol (DAG) in the ovaries. Tritiated palmitate tracing indicated reduced lipid transport to the ovaries. A significant reduction in hemolymph vitellogenin was also observed. These data suggest that T. rangeli impairs lipid mobilization and storage, thereby affecting egg production and reducing the insects' reproductive rate. **Supported by:** CAPES
Keywords: Rhodnius prolixus;Trypanosoma rangeli;Lipid.

HP - 088 - HISTOPATHOLOGICAL ANALYSIS OF CARDIAC CHAMBERS FROM PATIENTS WITH CHRONIC CHAGASIC CARDIOMYOPATHY (CCC)

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INTRODUCTION: Chronic Chagasic cardiomyopathy (CCC) affects 30% of patients from the chronic phase, and it is the leading cause of morbidity/mortality. Histopathological data obtained from distinct cardiac chambers from chronic Chagasic patients (CCPs) are scarce. **OBJECTIVES:** To compare the inflammatory infiltrate (I.I.) intensity and the myocardial fibrosis degree, in heart tissues from transplanted CCPs. **METHODS:** Heart tissue samples (right/left, atria and ventricles) obtained from 5 CCPs who underwent transplantation surgery at HC-UFMG (PII-PVI), and from 5 control donors (DII-DVI;left atrium/LA) were analyzed. Tissue sections (4–6 µm) stained with H&E and Masson's were evaluated across the all fields (10X) using a SQ scale (0-+4; absent to severe degrees). Mean values of inflammation/fibrosis were calculated for each chamber/patient. **RESULTS:** Predominantly lymphocytic infiltrates were most intense in the left ventricle (LV): 3/5 CCPs showed intense/severe inflammation (+3/+4), followed by the right atrium (RA; +2/+3 score). In the right ventricle (RV), 4/5 PCCs showed mild/moderate inflammation (+1/+2). Only two patients exhibited severe I.I. in the LA. Wide fibrosis areas were mostly observed in the RA chamber. Mild fibrosis (+1) was predominant in LA tissues, with moderate fibrosis (+2) observed only in one patient. Three CCPs exhibited moderate/intense fibrosis in the RV and LV. Among the controls, only DVI showed a small I.I. area, and moderate fibrosis(+2), which are rare findings in non-Chagasic individuals. **CONCLUSIONS:** LVs from PCCs exhibited the highest inflammation degree, followed by the RA; the LA was the lesser affected chamber. Fibrosis was larger in the RA and LV, along with the inflammatory scores, suggesting an association between inflammation and myocardial remodeling in CCCs. Future studies performed in a larger cohort are needed to confirm these findings, in addition to immunohistochemical characterization of tissue lymphocyte subpopulations.
Supported by: **Keywords:** Chronic Chagasic cardiomyopathy;Myocarditis;Fibrosis.

HP - 089 - 3D Biocurative Associated with a Zinc Complex for the Treatment of Tegumentary Ulcers Caused by Leishmania amazonensis: In vitro and In vivo Evaluation

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Leishmaniasis, classified by the WHO as a neglected tropical disease, affects approximately 1.3 million people annually and places 310 million individuals at risk of infection. Current therapies are associated with several adverse effects and contribute to the emergence of drug-resistant strains, highlighting the urgent need for alternative therapeutic approaches. Metalcomplexes, compounds containing a metal center capable of coordinating with organic ligands, offer advantages over conventional drugs by enabling multiple geometries and interactions. A zinc (II) complex was tested in vitro against Leishmania amazonensis promastigotes resulting in an IC50 of 0.98 to 1.8 µM after 24 and 120 h, respectively. Treated parasites presented ultrastructural alterations such as endoplasmic reticulum enlagement, plasma membrane blebbing and cytoplasm disintegration compatible with necrosis. Metalocomplex caused low toxicity to LLC-MK2 host cell line. In addition, we evaluated the in vivo effect of this metalocomplex associated with a biocurative. Lesions were induced in BALB/c mice (CEUA/UENF No. 486) and treated. After 40 days of treatment lesion size reduced, with the skin displaying epidermis, dermis, and hypodermis, indicating advanced regeneration compared with the control group. In vitro, the zinc (II) metallocomplex showed significant antileishmanial activity against Leishmania amazonensis promastigotes, with low toxicity toward LLC-MK2 host cells. In vivo, its association with the biocurative promoted lesion size reduction and advanced tissue regeneration after 40 days of treatment. These findings support the potential of this metalocomplex, particularly in combination with the biocurative, as a promising therapeutic strategy against cutaneous leishmaniasis. **Supported by:** CAPES **Keywords:** Biocurative;Leishmania amazonensis;ulcers.

HP - 090 - Immunochemotherapy Combining Chi-A/MPLA with Miltefosine Improves Therapeutic Outcomes in Experimental Visceral Leishmaniasis

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Visceral leishmaniasis (VL) is a severe neglected anthropozoonotic disease, mainly caused by Leishmania infantum in Brazil, with a fatality rate of up to 90% if untreated. The urban expansion of VL is closely related to the high prevalence of canine disease, which remains a major challenge for disease control. As the main domestic reservoir of L. infantum, infected dogs, including asymptomatic ones, play a key role in maintaining transmission. Limitations of current control measures and chemotherapy reinforce the need for safer and more effective therapeutic strategies for infected dogs. Immunotherapy and immunochemotherapy represent promising approaches by combining immune modulation with conventional antiparasitic treatment. This study investigated the therapeutic potential of Chi-A/MPLA-based immunotherapy and immunochemotherapy as prospective strategies for CVL. The Syrian golden hamster was selected as a preclinical model that closely reproduces progressive VL, strengthening the translational relevance to CVL. Hamsters were infected with L. infantum, treated 60-day post-infection, and therapeutic outcomes were assessed 30 and 90 days after treatment. Animals received Milteforan for 14 or 28 consecutive days. Chi-A/MPLA immunotherapy was administered either as two series of five doses separated by a 10-day interval or as two doses administered 28 days apart. Immunochemotherapy consisted of Chi-A/MPLA combined with a reduced 14-day Milteforan regimen. Hepatic biochemical changes were more evident at 30 days, with increased GGT and ALT, whereas AST remained comparable among groups. At 90 days, these alterations were less pronounced, with only increased AST in immunotherapy group. Histopathological analysis revealed hepatic granulomas at different maturation stages, including granulomas with parasitized multinucleated giant cells. Both therapeutic strategies reduced hepatic parasite burden at 30 days. At 90 days, parasite burden remained lower in treated animals, suggesting sustained parasite control and limited infection progression, with the highest reduction in the immunochemotherapy group. Overall, Chi-A/MPLA combined with a reduced 14-day Milteforan regimen showed the most favorable response, supporting immunochemotherapy as a strategy to improve parasite control while shortening chemotherapy exposure. Acknowledgements: UFOP, PROPPI, CCA-UFOP, CAPES, CNPq, FAPEMIG. **Supported by:** FAPEMIG – Fundação de Amparo à Pesquisa de Minas Gerais – APQ 02494-22; APQ-02511-22; APQ-02961-24; APQ-02835-25. CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico. INCT-DT – Instituto Nacional de Ciência e Tecnologia em Doenças Tropicais. CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior **Keywords:** Visceral leishmaniasis;Immunotherapy;Chemotherapy.

HP - 091 - The autophagic process in ovary of Rhodnius prolixus infected by *Trypanosoma rangeli*.

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Autophagy is an essential catabolic mechanism involved in cellular homeostasis through the degradation and recycling of cytoplasmic components. This process also participates in lipid mobilization through lipophagy, contributing to cellular energy metabolism. In the present study, we investigate the role of autophagy in the ovary of the insect *Rhodnius prolixus* during infection with *Trypanosoma rangeli*. Infection was performed by direct injection of 10^4 epimastigote-stage parasites into the hemolymph. Although previous studies have investigated the involvement of autophagy-related genes (ATGs) in triatomine insects, little is known about the role of this pathway during oogenesis in insects infected with trypanosomatids. Possible alterations in the expression of ATG1, ATG3, ATG6, and ATG8, which encode essential components of the autophagic machinery, were evaluated at 3 and 7 days post-infection by quantitative PCR (qPCR). Autophagic activity will also be investigated by Western blotting using an anti-ATG8 antibody, analysis of the ovarian lipid profile by thin-layer chromatography (TLC), and fluorescence microscopy using LC3 as an autophagosome marker. Preliminary results demonstrated a significant increase in ATG3 expression in the ovary at 3 days post-infection, whereas ATG1, ATG6, and ATG8 showed no significant differences compared with the control group. In the fat body, significant increases in ATG3, ATG6, and ATG8 expression were observed at 3 days post-infection. At 7 days post-infection, no significant differences were observed in the expression of the analyzed ATGs in the fat body, while ovarian ATG expression analyses at this time point have not yet been performed. Overall, these results suggest that *T. rangeli* infection is associated with tissue-specific alterations in the expression of components of the autophagic machinery in *R. prolixus*. Further analyses will help determine whether these alterations are associated with changes in the autophagic process and ovarian lipid metabolism.
Supported by: **Keywords:** Autofagia;Trypanosoma rangeli;Rhodnius prolixus.

HP - 092 - *Leishmania* non-coding RNAs large-scale screening to investigate regulatory functions at the host-parasite interface

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Leishmaniasis comprises a complex group of diseases caused by parasites of the genus *Leishmania*. In Latin America, most cases of cutaneous (CL) and mucocutaneous leishmaniasis (MCL) are caused by *Leishmania* species of the Viannia subgenus. Although genetic studies in *Leishmania* have traditionally focused on protein-coding genes and their roles in parasite development and differentiation, increasing attention has been directed toward the functional relevance of non-coding RNAs (ncRNAs) across different *Leishmania* species. To systematically investigate the contribution of ncRNAs to host–parasite interactions, we are implementing a high-throughput CRISPR/cas9strategy in which ncRNA genes, identified from the *L. guyanensis* transcriptome, are deleted and barcoded mutants pooled for phenotype analysis. We first developed a batch_sgRNA pipeline for large-scale CRISPR/Cas9 sgRNA design targeting predicted ncRNA genes. Using CCTop, 20-nt sgRNAs with a 3′-NGG PAM were selected based on predicted efficiency, off-target number and quality, and distance from the target locus. CRISPRater scores were also used to classify sgRNAs as having low, medium, or high predicted activity. The first 96 ncRNAs, preferentially expressed in amastigotes, and identified as conserved in the genomes of *L. braziliensis* and *L. guyanensis* have been submitted to the first round of gene deletion. Meanwhile, over the assembled genome of *L. guyanensis* RPL5, the transcriptomes of procyclic promastigotes, metacyclic promastigotes and amastigotes have been mapped to prioritise putative ncRNAs to be tested. This approach substantially increases the throughput and efficiency of functional screening, enabling the rapid identification of ncRNAs associated with fitness changes during parasite–host interactions. By combining pooled transfection with in vitro and in vivo infection models, this strategy provides a powerful framework for prioritising parasite ncRNAs with potential regulatory roles during mammalian infection.
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HP - 093 - Evaluation of the microbiota role in pathogenesis caused by Leishmania amazonensis

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Leishmaniasis comprises a group of neglected tropical diseases caused by protozoa of the genus *Leishmania*, presenting mainly as visceral and tegumentary forms. In Brazil, tegumentary leishmaniasis is the most prevalent and is commonly associated with species such as *L. amazonensis*, *L. braziliensis*, and *L. guyanensis*. This form is characterized by the development of cutaneous and/or mucosal lesions. The literature highlights the crucial role of $CD4^+$ T cells, particularly those associated with a pro-inflammatory Th1 response, in controlling *Leishmania spp* infection (Costa-da-Silva et al., 2022). In addition to their importance during the early stages of infection, as previously described by Soong et al. (1997), unpublished data from our group suggest that $CD4^+$ T cells also contribute to controlling the chronic phase of the disease. Previous studies have shown that dysbiosis can influence disease progression, promoting parasite elimination while worsening lesion severity (Naik et al., 2012). Based on these findings, this project aims to investigate the impact of the microbiota in a chronic model of *L. amazonensis* infection through the co-housing and co-nursing of wild-type (WT) mice with CD4 T cell-deficient ($CD4^{-/-}$) mice. Our preliminary data indicate that the microbiota derived from WT may play a protective role in lesion control in $CD4^{-/-}$ mice, preventing long-term lesion progression compared to $CD4^{-/-}$ control animals that did not undergo microbiota alteration induced by co-housing. Moreover, a higher prevalence of bacteria belonging to the genus Staphylococcus was observed in lesion samples. Further experiments are still required to better elucidate the role of the microbiota in cutaneous leishmaniasis. **Keywords:** Immune responses to parasitic diseases;Murine leishmaniasis;T Lymphocytes; Microbiota.

HP - 094 - Role of the cytokines IL-22 and IL-23 in Leishmania amazonensis infection in C57BL/6 mice

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Leishmaniasis are neglected tropical diseases caused by parasites of the genus *Leishmania*, manifesting primarily in cutaneous and visceral forms. Understanding the immunological mechanisms underlying the infection is essential for developing novel therapeutic strategies, as no specific vaccine is currently available. In this context, IL-22 is known to act predominantly in tissue repair, whereas IL-23 participates in sustaining an inflammatory response and driving effector cytokine production. Therefore, this study aimed to investigate the role of IL-22 and IL-23 during *Leishmania amazonensis* infection in C57BL/6 mice. To this end, independent experiments were conducted using wild-type (WT), IL-22 knock-out (KO), and IL-23 KO mice (average of five animals per group) infected intradermally with 2x106 *L. amazonensis* promastigotes. Lesion progression was monitored by caliper measurements, parasite burden was quantified by limiting dilution assay, and immune profiles were assessed by flow cytometry, alongside ongoing histological, immunohistochemical, and real-time PCR analyses. As a result, IL-22 KO mice exhibited significantly larger lesions and impaired wound healing, particularly during the chronic phase of infection, whereas IL-23 KO mice developed mild lesions comparable to those of WT controls. Furthermore, immunophenotyping revealed a compensatory enhancement of the cytotoxic response in the absence of IL-22, characterized by increased expansion and degranulation of CD8+, NK, and γδ T cells; however, this cytotoxic upregulation failed to reduce parasite load. In conclusion, IL-22 plays a crucial protective role in tissue resolution during *L. amazonensis* infection, while IL-23 exhibits a predominantly pro-inflammatory profile. Moreover, the heightened cytotoxic response observed in IL-22 deficiency does not improve parasite clearance and likely contributes to immunopathology by exacerbating tissue damage. **Supported by:** PIBIC-UFRJ **Keywords:** Leishmania amazonensis;citocinas;inflamação; imunologia; parasitologia;.