HP – 008 - **Characterization of a potential Fatty Acid-Binding Protein from Leishmania amazonensis**

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Leishmania parasites rely on host-derived lipids and fatty acids for energy metabolism, differentiation, immune modulation, and drug susceptibility. Yet the molecular mechanisms that govern lipid acquisition and utilization in *Leishmania* remain poorly understood. Calycins constitute a superfamily of β-barrel proteins that includes lipocalins and fatty acid-binding proteins (FABPs), both implicated in other organisms intracellular lipid transport. Bioinformatic analysis of the *Leishmania* genome revealed a conserved gene, here designated *lfabp1,* encoding a hypothetical protein with two putative calycin-like domains. The C-terminal domain (LeiFABP) was predicted to adopt an FABP-like fold. Recombinant LeiFABP was heterologously expressed, purified, and crystallized. X-ray diffraction data collected at the Manacá beamline (Sirius/CNPEM) enabled structure determination at 1.8 Å resolution, confirming the canonical FABP fold: ten antiparallel β-strands forming a closed β-barrel capped by a helix-loop-helix motif. Ligand-binding analyses by NMR indicated preferential interaction with dodecanoic acid (C12:0), a result corroborated by tryptophan-fluorescence assays. Polyclonal antibodies raised against recombinant LeiFABP were used to assess endogenous expression and subcellular localization. Western blot analysis detected LFABP1 in both promastigote and amastigote forms of *L. amazonensis*, while immunofluorescence revealed a predominantly cytoplasmic signal with signs of compartmentalization. To investigate the function of *lfabp1*, CRISPR-Cas9 gene editing was used to generate knockout and add-back strains now under characterization to elucidate LFABP1's role in the lipid metabolism of *L. amazonensis* and its impact on parasite pathogenicity.

Funding: Fapesp 2017/23933-3
Keywords: lipid;metabolism;knockout.

HP – 009 - **TcVPS23 acts in the biogenesis of extracellular vesicles and in the immunopathology in preclinical model of acute and chronic infection by *Trypanosoma cruzi***

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Chagas disease is caused by the protozoan *Trypanosoma cruzi*, resulting in severe inflammatory heart disease, stroke, arrhythmia, and sudden death. To date, the mechanism driving the development of Chagas cardiomyopathy has not been elucidated. The pathogenesis of this disease is multifactorial, which involves parasite virulence factors, the immune response, and the secretion of extracellular vesicles by *T. cruzi*. EVs shed by *T. cruzi* participate in cell-cell communication and can modulate the host immune response in favour of the parasite by inhibition of the pro-inflammatory response. However, the true role of *T. cruzi*-derived EV biogenesis in the development of Chagas immunopathology is unknown. Hence, we chose to delete the VPS23 gene, a component of the ESCRT-I machinery, codenamed TSG101 in mammalian cells, which acts as an important protein in exosome biogenesis. Our results show that VPS23 is essential in the biogenesis pathway of EVs shed by the bloodstream trypomastigote form (TCTs) of *T. cruzi* and acts as an important virulence factor. Low expression of VPS23 reduces the infectivity of parasites in myoblasts and cardiomyocytes, and this effect is reversed when these cells are pretreated with *T. cruzi–derived* secretome. In acute infection, low expression of VPS23 results in lower blood parasitemia, a smaller area of inflammation, and a reduction IFN-γ, IL6, and TGF-β levels. TGF-β is an anti-inflammatory cytokine that acts on the multiplication of *T. cruzi* in the acute phase and the development of cardiac fibrosis. In the chronic phase, animals infected with SKO TcVPS23 showed a decrease in the mast cell proliferation, cardiac fibrosis, and reduced levels of MCPI, KC, and IL6, chemokines and cytokines involved in mast cell activation. In the spleen, both in the acute and chronic phases, there was intense proliferation of CD3+CD4+IL17+ cells (Th17) and CD3+CD8+IL17+ (TcIL17) lymphocytes, and an increase in IL17 production via TcIL17. Th17 cells confer strong protective immunity against *T. cruzi*-related mortality, and IL17 is a critical factor in sustaining CD8+ T lymphocyte cellular immunity against the parasite. These findings demonstrate that VPS23, the ESCRT-I component, acts in the release of EVs and soluble molecules that alter the immune response of the host, and consequently, the development of myocardial fibrosis in the *T.cruzi*-infected host.

Supported by: FAPESP
Keywords: Vesicles ;VPS23 ;Trypanosoma cruzi .

HP – 010 - **Characterization of a potential Fatty Acid-Binding Protein from *Leishmania amazonensis***

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Leishmania parasites rely on host-derived lipids and fatty acids for energy metabolism, differentiation, immune modulation, and drug susceptibility. Yet the molecular mechanisms that govern lipid acquisition and utilization in *Leishmania* remain poorly understood. Calycins constitute a superfamily of β-barrel proteins that includes lipocalins and fatty acid-binding proteins (FABPs), both implicated in other organisms intracellular lipid transport. Bioinformatic analysis of the *Leishmania* genome revealed a conserved gene, here designated *lfabp1,* encoding a hypothetical protein with two putative calycin-like domains. The C-terminal domain (LeiFABP) was predicted to adopt an FABP-like fold. Recombinant LeiFABP was heterologously expressed, purified, and crystallized. X-ray diffraction data collected at the Manacá beamline (Sirius/CNPEM) enabled structure determination at 1.8 Å resolution, confirming the canonical FABP fold: ten antiparallel β-strands forming a closed β-barrel capped by a helix-loop-helix motif. Ligand-binding analyses by NMR indicated preferential interaction with dodecanoic acid (C12:0), a result corroborated by tryptophan-fluorescence assays. Polyclonal antibodies raised against recombinant LeiFABP were used to assess endogenous expression and subcellular localization. Western blot analysis detected LFABP1 in both promastigote and amastigote forms of *L. amazonensis*, while immunofluorescence revealed a predominantly cytoplasmic signal with signs of compartmentalization. To investigate the function of *lfabp1*, CRISPR-Cas9 gene editing was used to generate knockout and add-back strains now under characterization to elucidate LFABP1's role in the lipid metabolism of *L. amazonensis* and its impact on parasite pathogenicity.
Supported by: FAPESP (2023/02831-9)
Keywords: Leishmania;lipid metabolism;CRISPR-Cas9.

HP – 011 - **Sulphur-Containing Amino Acid Metabolism in Trypanosoma cruzi: Functional Insights from Gene Knockout and NMR-Based Metabolomics**

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Trypanosoma cruzi, the causative agent of Chagas disease, relies on amino acid metabolism to sustain its complex life cycle. Among these, sulphur-containing amino acids—cysteine (Cys) and methionine (Met)—play key roles in redox balance, energy metabolism, and methylation reactions. To explore their metabolic relevance, total or partial knockout (KO) lines were generated for five key enzymes: SAT and CS (involved in *de novo* Cys biosynthesis), and AMS, AHCH, and HMT (involved in the Met trans sulphuration cycle). The impact of gene deletion on redox resistance, benznidazole susceptibility, and metabolic output was assessed.
KO lines for AMS, AHCH, and HMT exhibited increased sensitivity to H_2O_2, indicating impaired redox homeostasis. These same mutants also showed lower IC_{50} values for benznidazole, suggesting that disruption of sulphur metabolism may compromise thiol-dependent detoxification mechanisms. ^{1}H-NMR exometabolomics of KO lines incubated with single carbon sources (Cys, Met, Ser, or glucose) under nutritional stress (NS) revealed significant alterations in metabolite excretion profiles. Notably, $HMT^{+/-}$ mutants showed elevated excretion of acetate and formate under NS, suggesting rerouting of metabolic fluxes due to impaired Met regeneration. $SAT^{-/-}$ mutants displayed increased acetate and formate release upon incubation with Cys and Ser, implicating a compensatory activation of the trans sulphuration pathway. In contrast, $CS^{+/-}$ mutants exhibited marked reduction in acetate and formate excretion, reinforcing CS's key role in sulphur amino acid metabolism. These changes were consistent with redox sensitivity and increased benznidazole susceptibility in selected lines. These findings highlight the central role of sulphur amino acid pathways in *T. cruzi* metabolism and redox regulation and support their potential as therapeutic targets to complement benznidazole therapeutics.
Supported by: FAPESP: grant to AMS N° 2021/12938-0, grant to MSB N° 2022/14959-7
Keywords: Trypanosoma cruzi;metabolomics;sulphur amino acids.

HP – 012 - **Exometabolomic Analysis of Branched-Chain Amino Acid Catabolism Disruption in Trypanosoma cruzi**

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Chagas disease, caused by the protozoan *Trypanosoma cruzi*, continues to be a significant public health challenge in the Americas. *T. cruzi* displays notable metabolic flexibility, utilizing amino acids not only as an alternative to glucose for carbon and energy but also for critical biological functions, such as life cycle transitions, stress adaptation, and host cell invasion. In this work, we explored how various metabolic pathways in *T. cruzi* interact with the branched-chain amino acid (BCAA: leucine, isoleucine, and valine) catabolic pathway and affect cellular processes. For this purpose, we analyzed the exometabolome, the secretion of metabolic byproducts, by comparing knockout strains of specific enzymes with the wild-type and parental (Cas9) strain. Using ^{1}H-NMR-based exometabolomics, we examined knockout strains lacking enzymes in the BCAA degradation pathway, including components of the branched-chain α-keto acid dehydrogenase complex (E1, E2, E3), isovaleryl-CoA dehydrogenase (IVDH), and enoyl-CoA hydratase (ECH). Notable observations included elevated secretion of propylene glycol and formate (e.g., in E3a KO and IVDH KO strains), decreased acetone release (e.g., in E1α KO and IVDH KO strains), and modified levels of isobutyrate and 2-hydroxyisovalerate in the E1α KO strain cultured with valine. While most changes corresponded to the disrupted pathways, some metabolites, like formate and propylene glycol, exhibited unexpected shifts, hinting at possible cross-pathway interactions or adaptive responses that warrant further study. These results highlight the metabolic interconnectedness of amino acid pathways in T. cruzi and emphasize the biological importance of BCAA degradation enzymes. This understanding could contribute to the discovery of new drug targets against the parasite.
Supported by: FAPESP: grant to AMS 2021/12938-0, grant to SM 2022/09116-0
Keywords: Trypanosoma cruzi;Branched-chain amino acid metabolism;Exometabolomics.

HP – 013 - **Generation of miltefosine-resistant lines in Leishmania infantum: Exploring the role of the miltefosine - sensitive locus**

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Leishmania infantum is the etiological agent of visceral leishmaniasis (VL), a parasitic disease endemic in South America that can lead to death if not treated. Miltefosine (MF) is the only oral drug approved for the treatment of VL. In Brazil, the deletion of the miltefosine-sensitive *locus* (MSL), located on chromosome 31 of *L. infantum* isolates from Brazilian patients was associated with treatment failure to MF and low responsiveness *in vitro*. Due to these findings, MF is not considered as alternative to treat VL patients in Brazil. The MSL contains four genes: 3'-nucleotidase/nucleases (NUC1 and NUC2); helicase (HLP); and 3,2-trans-enoyl-CoA isomerase (TEI). In this study, we evaluated the MF susceptibility *in vitro* of *L. infantum* strains and clinical isolates that contain or not this *locus* (MSL positive and negative respectively). Our findings showed that MF susceptibility was similar among all strains and isolates evaluated, suggesting that MSL may not be directly involved in MF resistance. To further evaluate the impact of MSL in the response to MF *in vitro,* we generated MF-resistant lines using MSL positive and negative strains. Resistant lines were generated by two different protocols: by chemical mutagenesis and by increasing drug pressure at promastigote stage. MF-resistant clonal lines were obtained using both protocols, despite the presence or not of MSL. No structural change in the MSL was found in MF-resistant lines selected by these two methods of selection. We also tried to generate resistant lines at the intracellular amastigote stage, by exposing MSL positive and negative parasites to sublethal concentrations of MF for more than ten passages. However, no resistant line was obtained using this protocol. We plan to resequencing the genome of these MF resistant lines to evaluate whether MSL and/or other genes are involved in the mechanism of MF resistance in MSL positive and negative strains of *L. infantum*. In addition, the role of MSL in MF response will be also evaluated *in vivo*, using an animal model susceptible to *L. infantum* infection.
Supported by: Fapesp
Keywords: Leishmania infantum;drug resistance;miltefosine.

HP – 014 - **From Phosphonate to Pathogenicity: 2AEPPT Drives AEP Utilization and Parasite Fitness in *T. cruzi***

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The surface of *Trypanosoma cruzi* is densely coated with glycoconjugates including glycoproteins anchored via glycosylphosphatidylinositol (GPI), which play key roles in parasite protection and chronic infection establishment. Among the unusual components of these surface molecules is 2-aminoethylphosphonate (AEP), a natural phosphonate found in glycosylinositolphospholipids (GIPLs) of *T. cruzi* and other trypanosomatids. A gene encoding a putative 2-aminoethylphosphonate pyruvate transaminase (2AEPPT EC 2.6.1.37), which catalyzes the first step in AEP catabolism, is has been identified in the genome of *T. cruzi*. Despite its predictable relevance, the role of 2AEPPT in the parasite biology has not been determmined. Here, we report the functional characterization of *Tc*2AEPPT. We isolated the full-length gene from the CL Brener strain, confirmed its enzymatic activity through recombinant expression in *E. coli*, and generated 2AEPPT knockout epimastigotes using CRISPR-Cas9. In the KO parasites, we observed a ~40% reduction in epimastigote proliferation compared to the parental line. Moreover, infection assays in mammalian cells showed a marked decrease in trypomastigote release, suggesting impaired differentiation or egress. Remarkably, addition of AEP to the culture medium restored the proliferation phenotype, strongly supporting a direct role for this metabolic pathway in the parasite viability. Considering that AEP is a structural component of surface GPIs, these findings support that 2AEPPT activity is essential for GPI biosynthesis, membrane remodeling, and ultimately parasite fitness and infectivity. This enzyme represents a central metabolic node linking phosphonate utilization to survival and persistence in both insect and mammalian hosts. Due to its absence in mammals and unique presence in protozoans, 2AEPPT is a promising candidate for antiparasitic drug development.

Supported by: FAPESP 2023/ 06791-1
Keywords: Trypanosoma cruzi;2-aminoethylphosphonate Pyruvate, Transaminase;glycosylphosphatidylinositol.

HP – 015 - **Immunization with *Trypanosoma cruzi* Trans-sialidase containing SAPA repeats protects mice against infection and promotes a balanced inflammatory response**

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Proteins containing repeat domains are widespread among protozoa parasites. *Trypanosoma cruzi*, the causative agent of Chagas disease (CD), has a family of surface proteins named trans-sialidases (TS), responsible for transferring sialic acid from host glycoconjugates to parasite mucins. Some TS have a C-terminal domain containing 12 amino acids repeats known as SAPA (shed acute parasite antigen). **Epitope prediction** shown that C-terminal SAPA domain contains mostly B-cell epitopes and it has been shown that the SAPA repeats increase the stability of the enzyme TS in the blood, which is considered a parasite virulence factor. Shed in the blood of the infected host, TS mediates several biological effects and because of its essential role during infection, it has been tested recurrently as a vaccine candidate against CD. Here, we investigate the effect of immunizing mice with recombinant TS proteins with and without (w/o) SAPA repeats, as well as with a protein containing only the repeat domain. Besides confirming the immunodominance of the SAPA domain, after challenging immunized animals with *T. cruzi*, we showed that the presence of the repeats did not significantly impact protection and parasite numbers after infection. However, immunization with TS protein containing the repeat domain resulted in increased production of interleukin-10 (IL-10) compared to mice immunized with TS w/o SAPA, and this increased IL-10 production correlates with a significant reduction in the inflammatory infiltrate in heart tissues of infected animals. The results indicate that the immunodominant SAPA domain plays a role in promoting an anti-inflammatory response that may favor parasite survival. On the other hand, as a vaccine component, the presence of SAPA repeats may contribute to promoting a desirable, more balanced immune response.

Supported by: CAPES, FAPEMIG, CNPq and INCTV
Keywords: Chagas Disease;Vaccine;Trans-sialidase.

HP – 016 - **Invariant Surface Glycoproteins of *Trypanosoma evansi:* In Silico Epitope Mapping and Implications for Equine Surra Immunity**

COUTINHO, C.D.C.; LEFKADITIS, A.P.C.; MENDES, A.C.; DA SILVA, I.A.M.; AZEVEDO, G.M.; NÁJERA, C.A.; BAHIA, D..
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Trypanosoma evansi is the etiological agent of Surra, a veterinary disease causing severe clinical signs in equines. Understanding the host immune response is valuable for insights into host-specific mechanisms and for supporting prophylactic strategies against disease progression. In this context, 65 kDa Invariant Surface Glycoproteins (ISG65s) are noteworthy, being linked to immune recognition and evasion in *T. brucei*, yet little is known about them in *T. evansi*. Thus, this study aimed to characterize *T. evansi* ISG65s and identify conserved epitopes relevant to adaptive immune response in horses with Surra. The predicted proteome of T. evansi STIB805 was retrieved from TriTrypDB v.68. ISG65s were analyzed to predict signal peptides, transmembrane domains, GPI anchors and subcellular localization using several bioinformatic tools. Linear B-cell and MHC I epitopes were predicted with ABCPred and NetMHCpan 4.1, respectively. Proteins and epitopes were further evaluated for antigenicity and toxicity with VaxiJen 2.0 and ToxinPred. Molecular docking of predicted epitopes and MHC I alleles was performed in the HDOCK server. Eight 65 kDa ISGs were identified in the predicted secretome of *T. evansi*, five located on chromosome 2 and three on chromosome 5. In total, 91 B-cell epitopes were predicted. Of these, 25 MHC I epitopes showed strong predicted binding affinity. Fourteen were identified as antigens, of which nine were found to be immunogenic and non-toxic. Notably, one epitope demonstrated the optimal docking score with allele Eqca-100101 and was identified in ISGs located on chromosome 2. Another epitope demonstrated the optimal docking score with Eqca-1600101 and was exclusive to a single glycoprotein from chromosome 5. These findings underscore the potential for the development of equine-specific vaccines or immunotherapies against Surra.

Supported by: FAPEMIG (APQ-00872-23), CNPq and CAPES
Keywords: ISGs;immune response;reverse vaccinology.

HP – 017 - **Protein acetylation dynamics in mammalian hosts during *Leishmania* infection.**

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Eukaryotic cells use post-translational modifications such as lysine acetylation to regulate gene expression and the immune response, playing a crucial role in cellular homeostasis. Lysine acetylation levels are critical for chromatin condensation and are regulated by lysine deacetylases (KDACs), which remove acetylation marks to favor chromatin condensation and gene downregulation. *Leishmania* spp. infection induces macrophage polarization, favoring a phenotype that subverts immune response, decreasing pro-inflammatory cytokine production and microbicidal capacity. This study investigates the role of different KDACs during *Leishmania* infection. The deacetylase family comprises 18 proteins: 7 sirtuins (NAD^+-dependent deacetylases, class III), and 11 Zn^{2+}-dependent HDACs (classes I, IIa, IIb, and IV). Three public GEO datasets were analyzed for human deacetylase expression during infection with *L. braziliensis* and *L. infantum*. Curated data showed several KDACs differentially expressed in tissue and blood from infected patients. Notably, expression of class I HDACs, especially HDAC3 and HDAC8, significantly increased during chronic infection. Additionally, peripheral blood samples from patients diagnosed with cutaneous leishmaniases before and after clinical cure were evaluated by qPCR for HDAC2, HDAC3, HDAC6, and HDAC8, showing expression changes in class I and II KDACs. *In vitro* infection of macrophages with *L. amazonensis* also revealed different global protein acetylation levels compared to non-infected cells. Thus, preliminary data suggest that acetylation regulation in host-parasite interactions is crucial for *Leishmania* infection establishment and may reveal novel therapeutic targets, offering new insights and innovative approaches for treating leishmaniasis, which affects millions globally.

Supported by: FAPESP: 2025/06986-2
Keywords: Leishmania infection;lysine deacetylases;protein acetylation.

HP – 018 - **Insights into T. cruzi Life-stage Metabolism Through Genome-scale Metabolic Modeling**

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Trypanosoma cruzi, the parasite causing Chagas disease, has three main life cycle stages: epimastigote, amastigote and trypomastigote, which are adapted to the different environments (insect gut, bloodstream, host cytoplasm) they live. In several of these environments, nutrients are limited. The parasite's survival depends on active transporters and enzymatic machinery at each life stage, enabling available nutrients uptake and processing. Understanding the metabolic diversity across T. cruzi's life cycle could provide key insights into its biology, potentially revealing essential enzymes as drug targets and elucidating metabolic reprogramming mechanisms. Currently, systems biology approaches have been employed for in silico metabolic reconstructions of various organisms, allowing systemic studies of metabolic flux through computational simulations. Experimental data—such as culture media, metabolite uptake/secretion rates, and multi-omics datasets—can be integrated into genome-scale metabolic models (GEMs) to constrain and refine simulations, improving their predictive accuracy. This study aims to use the T. cruzi iTc791 metabolic model for computational reconstruction of the parasite's metabolism across its three life stages. With this approach we generated three stage-specific GEMs of T. cruzi with predictive power. Our models include 648 genes, 1132 reactions and 1042 metabolites (Epimastigote), 516 genes, 979 reactions, 1001 metabolites (Amastigote) and 499 genes, 962 reactions and 987 metabolites (Trypomastigote). The Flux Balance Analysis (FBA) showed different metabolic flux distributions in the glycolytic pathway, oxidative phosphorylation and fatty acid oxidation between stages and in the metabolic excretion profile. With these models it is possible to simulate distinct conditions of the parasite life's cycle and culturing conditions. Omics dataset are being used to improve the model as a tool for in silico metabolic studies of T. cruzi.

Supported by: 2023/07306-0
Keywords: Systems Biology;Metabolism;Genome-scale metabolic models.

HP – 019 - **Characterization of the expression and localization of two cdc2-related kinases during the cell cycle and metacyclogenesis of T. cruzi.**

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The cell cycle is a conserved and tightly regulated process in model eukaryotes, with control exerted by cyclin-dependent kinases (CDKs), which also play a role in cell differentiation. In *Trypanosoma cruzi*, the protozoan responsible for Chagas disease, various cellular changes occur throughout the cell cycle and life cycle. Although the signals triggering transitions between developmental forms are still poorly understood, cell cycle regulation in this trypanosomatid is mediated by Cdc2-related kinases (CRKs). Therefore, the main objective of this study was to investigate the expression and localization of CRK1 and CRK3 during the cell cycle and metacyclogenesis of *T. cruzi*. CRISPR/Cas9 was used to generate strains expressing CRKs tagged with mNeonGreen and Myc. Expression was assessed by Western blotting, hydroxyurea was used to synchronize the cell cycle and metacyclogenesis was performed in TAU 3AAG medium. Localization was determined by immunofluorescence using anti-Myc antibodies, and protein quantification and statistical analysis were performed using Photoshop and GraphPad Prism v.8. The results showed that both CRKs are expressed throughout the parasite's cell cycle, with no significant variations on expression levels. However, during the transition to the non-replicative metacyclic trypomastigote forms, differences were observed between the kinases. While CRK1 remain expressed after metacyclogenesis, the expression of CRK3 was significantly reduced at the end of metacyclogenesis. Regarding subcellular localization during the *T. cruzi* cell cycle, CRK1 is distributed in the cytoplasm, with no nuclear localization at any stage, and mitochondrial localization throughout the cycle. CRK3 is found in the nucleus during G1, relocates to the cytoplasm in S, G2, and mitosis, and becomes concentrated near the nucleus during cytokinesis. In metacyclic trypomastigote forms, CRK1 remained cytoplasmic, while CRK3 was not detected. In conclusion, the constitutive expression of the CRKs analyzed in *T. cruzi* is consistent with the profile observed for CDKs in other eukaryotes, and the differences in their subcellular localization patterns suggest that these kinases may perform distinct functions in the parasite. Moreover, the presence of CRK1 in metacyclic trypomastigotes raises the question of whether this kinase retains catalytic activity or remains poised for cell cycle reactivation during the transition to the amastigote form.

Supported by: FAPESP: 2022/08866-6
Keywords: CRKs;differentiation;trypanosomatid.

HP – 020 - **Characterization of a Serine/Threonine Protein Kinase in the proteome of two Trypanosoma cruzi strains with different infectivity.**

AZEVEDO, G.M.; MENDES, A.C.; LEFKADITIS, A.P.C.; COUTINHO, C.D.C.; DA SILVA, I.A.M.; NÁJERA, C.A.; BAHIA, D..
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Protein kinases (PKs) are responsible for the reversible phosphorylation of protein substrates via cellular regulatory mechanisms. This leads to conformational changes that modify enzymes, specific gene transcription, and protein degradation. Despite the activation of cellular signalling pathways in response to parasite invasion of the host, only a limited number of PKs have been characterised in T. cruzi. In this study, we characterized and validated a serine/threonine protein kinase (STPK) modulated in the *T. cruzi* proteome. Protein extracts obtained from extracellular amastigote forms of CL (less infective) and G (more infective) strains were processed by LC-MS/MS. STPK exhibited positive modulation in CL strain compared to G strain. Bioinformatics tools were used to search for signal peptides, transmembrane regions, GPI anchors, and cellular localization. Additionally, *in silico* analyses also suggested possible interactions with nuclear proteins, indicating involvement in regulatory processes. Conserved domains were identified using ScanProsite, and the most hydrophilic region was detected by ProtScale for antibody production. The entire gene and the regulatory domain were then cloned and expressed in bacteria. Western blot analysis indicated a higher protein abundance in the CL strain. This finding was consistent with the proteomic data. Consequently, the hypothesis that the STPK protein is associated with the infectivity of the parasite warrants further investigation. Gene deletion is currently being investigated using CRISPR-Cas9 technology, with a view to confirming its biological function and relevance in the parasite cell cycle. The present study proposes STPK as a potential target for inhibitory drugs that can neutralise the parasite's infectivity and intracellular development.
Supported by: FAPEMIG - 6.31/2021; CNPq, CAPES.
Keywords: Serine-threonine protein kinase;Trypanosoma cruzi;Total proteome.

HP – 021 - **IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF A DENSE GRANULE PROTEIN ASSOCIATED WITH CYSTS OF *Neospora caninum***

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Neospora caninum is an obligate intracellular parasite and the pathogenic agent of neosporosis, a leading cause of abortions in cattle worldwide. During the infection, specialized secretory organelles known as dense granules release a group of proteins (GRAs) that promote intracellular parasitism and modulate the host immune response. Although GRAs have been extensively studied in *Toxoplasma gondii*, their roles in *N. caninum* remain largely unexplored. Investigating these proteins may uncover novel immunomodulatory functions or vaccine targets, and provide insights into the parasite's mechanisms of immune evasion. Through transcriptomic analysis and BioID assays, we identified a novel GRA in *N. caninum*, that has an orthologue previously associated with the cyst structure of *T. gondii*. To characterize its function, we employed the CRISPR/Cas9 system for endogenous tagging, followed by gene disruption. The protein expression and molecular weight of the protein was confirmed by Western Blot, and immunofluorescence assays revealed localization of the novel GRA within the parasitophorous vacuole of tachyzoites. Phenotypic characterization of the knockout strain via plaque assays showed marked morphological alterations. *In vivo* experiments using murine models demonstrated that parasites lacking the targeted GRA exhibited reduced parasite burden in the peritoneal cavity during acute infection (24h), as well as decreased parasite load in the brain during chronic infection (30 days), when compared to the wild-type strain (NcLiv). These findings highlight the crucial role of GRAs in parasite replication and immune evasion. Accordingly, deeper understanding is essential for advancing the development of effective vaccines and therapeutic strategies against neosporosis.
Supported by: CAPES 88887.007321/2024-00
Keywords: GRAs;Neospora caninum;Cyst.

HP – 022 - **Identification and Functional Characterization of a Novel GRA Protein in *Neospora caninum* Through CRISPR/Cas9-Mediated Gene Editing.**

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Neospora caninum is an obligate intracellular apicomplexan parasite that is a major cause of abortion in cattle. Its close relative, *Toxoplasma gondii*, has been more extensively studied, especially regarding secreted effectors from organelles such as the dense granules (GRAs), which are key molecules to remodel the parasitophorous vacuole (PV), acquiring nutrients, and to modulate host immunity. Despite their partial conservation in Apicomplexa, GRAs in *N. caninum* remain poorly characterized. This study aimed to identify and functionally characterize a novel GRA in *N. caninum*, focusing on parasite virulence and immune modulation. BioID-based proximity labeling was performed using a known *T. gondii* GRA fused to BirA* to biotinylate PV-associated proteins in *N. caninum*, which were later sequenced by LC-MS/MS. Dozens of new *N. caninum* GRA proteins were sequenced and we selected our target based on transcriptomic presence, predicted conserved domains, and orthology to *T. gondii* GRAs. Our candidate was endogenously HA-tagged to confirm PV localization by Immunofluorescence Assay. Protein expression and molecular weight were determined by Western Blotting. We used CRISPR/Cas9-mediated gene disruption to assess its function. Knockout parasites showed significantly reduced plaque formation, suggesting a possible involvement in intracellular survival or replication. Infection of bone marrow-derived macrophages (BMDMs) revealed altered cytokine profiles during early infection, and *in vivo* studies indicated immunomodulatory effects despite stable parasite burden. These findings show that this novel GRA could contribute to immune modulation during the acute infection, providing a new target for therapeutic or prophylactic interventions against neosporosis.
Supported by: FAPEMIG, CAPES, CNPq
Keywords: GRAs;Neospora caninum;Host-Pathogen Interaction.

HP – 023 - **Identification and molecular characterization of microneme proteins in the activation of the immune response by *Neospora caninum* infection**

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Neospora caninum is an obligate intracellular parasite of the phylum Apicomplexa, responsible for neosporosis, a worldwide spread disease affecting dogs and cattle. Parasites of this phylum are notable for having an apical complex composed of proteins that are crucial for the adhesion and invasion of host cells, processes that are essential for the development of acute infection by this parasite. However, these proteins have been poorly studied. This study evaluates the role of microneme protein in activating the immune response during *N. caninum* infection. To this end, we performed transcriptomic analyses and BioID assays and identified potential microneme proteins for this study. In order to study those targets, we used the CRISPR-Cas9 technology to genetically modify tachyzoites by endogenous tagging and gene deletion. The endogenous tag demonstrated that those predicted proteins localized to the micronemes, and we also analyzed its protein expression profile by Western Blotting assays. Plaque assays with knockout parasites showed that the MIC proteins were required for proper parasite proliferation *in vitro*. *In vivo* assays with mice experimentally infected with WT and knockout parasites showed increased parasite load during the acute phase of the infection (24 hours), while the opposite results were observed in the brain tissue during chronic infection (30 days), when a lower parasite burden was observed in mice infected with the knockout parasites. This study underscores the importance of micronemal proteins in the biology of *N. caninum* and highlights their potential as targets for vaccine development and therapeutic interventions against neosporosis.
Supported by: CAPES - 88887.949081/2024-00
Keywords: Neospora caninum;microneme proteins;CRISPR-Cas9.

HP – 024 - **Characterization of Cyclin 5 and Wee1 proteins in Trypanosoma cruzi: Shared conserved features with eukaryotic cell cycle regulators**

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The cell cycle is a meticulously regulated process that ensures precise DNA replication and cell division. While there is significant conservation of these elements across eukaryotes, species-specific variations emerge from the addition or replacement of proteins or alterations in regulatory pathways. In the classical model, CDKs are activated by cyclins and inhibited by regulators such as WEE family kinases. In *Trypanosoma cruzi*, the etiological agent of Chagas disease, cell cycle regulation is imperative for the transition between proliferative, non-infective forms (epimastigotes and amastigotes) and infective, non-proliferative forms (metacyclic and bloodstream trypomastigotes). However, the underlying molecular mechanisms remain poorly understood. *T. cruzi* encodes CRKs (Cdc2-related kinases) and cyclins, but their interactions and roles in specific cell cycle stages are not fully elucidated. Canonical CDK inhibitors have not been identified, except for a WEE1 homolog described in three trypanosomatids, including *T. cruzi*. This study aimed to characterize Cyclin 5 and Wee1, assessing their expression during the cell cycle and metacyclogenesis, and their subcellular localization. Using CRISPR/Cas9, c-Myc and mNeonGreen were inserted at the 5' region of Wee1, and c-Myc at the 3' region of Cyclin 5. Edits were validated by growth curves, PCR, Sanger sequencing, and western blotting. Hydroxyurea synchronization showed differential expressions of both proteins, with peaks in S phase. During metacyclogenesis, Cyclin 5-Myc decreased but remained detectable, while Wee1-Myc was absent, as confirmed by immunofluorescence. These findings reveal similarities with model eukaryotes, such as oscillatory CDK regulator expression, non-canonical cyclin roles, and nuclear redistribution of Wee1 during mitosis. Clarifying these mechanisms is crucial to understanding how cell proliferation adapts across the life cycle, contributing to developmental plasticity and infectivity.

Supported by: CNPq: 177421/2024-0/ FAPESP: 2024/23848-0
Keywords: Kinase regulation;Endogenous protein tagging;Trypanosomatidae.

HP – 025 - **Deletion of the glucosamine-6-phosphate N-acetyltransferase gene in *Trypanosoma cruzi* and its impact on parasite biology**

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Trypanosomatids are rich in surface glycoconjugates, molecules that are essential for the survival, infectivity, and virulence of these parasites. Hexosamines link hexoses and amino acids metabolism to the synthesis of nucleotide sugars via the hexosamine biosynthesis pathway (HBP), which provides substrates for glycosylation reactions. Glutamine (Gln), a key amino acid for *Trypanosoma cruzi*, acts as an amino group donor in the biosynthesis of these glycoconjugate precursors. This study focuses on the relationship between Gln and amino sugar production. Here, we investigate the role of glucosamine-6-phosphate-N-acetyltransferase (GNA; EC 2.3.1.4), which catalyzes the second step of the pathway connecting Gln to the HBP. To this end, the *Tc*GNA gene encoding the *T. cruzi* GNA was deleted using the CRISPR/Cas9 system. Genotyping revealed that $GNA^{-/+}$ mutant lines exhibit an average reduction of 56% in mRNA levels, confirming the partial deletion of the target gene. This reduction impaired the proliferation of epimastigotes *in vitro* during the exponential growth phase compared to the control. The ability of $GNA^{-/+}$ epimastigotes to differentiate into metacyclic trypomastigotes was also assessed in comparison to the parental cell line. The generation of null mutant lines for the GNA gene, as well as for other genes encoding enzymes that utilize Gln, will help to elucidate the importance of this metabolite for cell viability and for the synthesis of surface glycoconjugates. These findings will provide new insights into the role of glutamine in the hexosamine biosynthesis pathway in parasite biology, and serve as a foundation for future therapeutic strategies and control approaches for Chagas disease.

Supported by: FAPESP 2023/11104-3
Keywords: T; cruzi;Glucosamine-6-phosphate-N-acetyltransferase;CRISPR/Cas9.

HP – 026 - **Comparative secretome between Trypanosoma brucei gambiense and Trypanosoma evansi**

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The Trypanosomatidae family, which comprises a variety of protozoa, is responsible for the transmission of numerous diseases affecting humans and animals. A number of these species represent the primary focus of research, including *T. brucei gambiense* (Tbg), and *T. evansi* (Tev). Comparative studies between Tbg and Tev revealed that these species are closely related phylogenetically, raising questions regarding their infection mechanisms. The aim of this research is to ascertain the underlying reasons for the observed discrepancy in the target-hosts of these closely related species: while Tbg infects predominantly humans, Tev infects exclusively animals. An in silico survey of secreted and membrane proteins is currently underway. These proteins are of critical importance in the context of host-pathogen interaction. Both Tbg DAL972 and Tev STIB805 proteomes were retrieved from the TriTrypDB database. The secretome analysis was conducted by bioinformatics tools such as SignalP 6.0 and DeepTMHMM 1.0. OrthoVenn3 has been used to identify ortholog clusters between the two species, resulting in the prediction of 33 ortholog groups between Tev and Tbg, with the majority of the proteins being characterised as hypothetical. Exclusive proteins were identified for each of the targeted species. For Tbg, the majority of the exclusive ortholog clusters were found to be associated with hypothetical and Invariant Surface Glycoproteins (ISG). In the case of Tev, the exclusive proteins were predominantly related to cysteine peptidase and Variant Surface Glycoproteins (VSG), in addition to a Heat Shock Protein (HSP). The results of this study could provide valuable insight into the reasons why these closely related species have different hosts, as well as help to identify the most promising pharmacological target for further research.

Supported by: FAPEMIG (APQ-00872-23), CNPq, CAPES
Keywords: T; evansi;T; b; gambiense;proteomic.

HP – 027 - **EFFECTS OF PEPTIDASE INHIBITORS ON *STRIGOMONAS CULICIS* GROWTH AND SURVIVAL**

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Strigomonas culicis is a monoxenic trypanosomatid that harbors a bacterial endosymbiont, which plays a crucial role in the parasite's energy and oxidative metabolism. The comparison between the wild-type (WT) and aposymbiotic (APO) strains provides an excellent model to explore endosymbiosis influences on parasite physiology. through peptidase expression, especially cysteine- and metallopeptidases such as calpains, cruzipain, and GP63. This study aimed to compare the effects of proteolytic inhibitors against cysteine- and metallopeptidases on the growth and survival of the symbiont-harboring and APO strains of ***S. culicis***. Cell proliferation assays using the calpain inhibitor MDL-28170 resulted in a reduced growth, particularly after 72h, with IC_{50} values of 55 µM for the WT and 19 µM for the APO strain. Additional viability assays using Presto Blue® confirmed 1,10-phenanthroline cytotoxicity at 100 µM, allowing to state non-lethal doses for future adhesion assays. Triatomines were infected with the WT strain to obtain recently isolated (RI) parasites. The peptidase expression of RI was analyzed by flow cytometry using antibodies against GP63, calpains, and cruzipain. Remarkably, an increased calpain expression was observed in RI parasites, while the APO strain showed elevated GP63 levels. Collectively, these findings suggest that peptidases, especially GP63 and calpains, are critical in ***S. culicis*** growth and survival. Ongoing experiments will evaluate the effect of peptidase inhibitors on parasite adhesion to insect intestines, contributing to a better understanding of virulence regulation in monoxenic trypanosomatids.

Supported by: Fundação Carlos Chagas Filho de Amparo à Pesquisado Estado do Rio de Janeiro - E-26/210.976/2024
Keywords: trypanosomatids;flow cytometry;parasite adhesion.

HP – 028 - **Characterization of cysteine catabolism via mercaptopyruvate sulfurtransferase in Trypanosoma cruzi**

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Amino acids play vital roles in *Trypanosoma cruzi* biology, not only supporting protein synthesis but also participating in other critical processes, such as differentiation, host cell invasion, and stress adaptation. In the cysteine catabolism, aspartate aminotransferase (ASAT) generates 3-mercaptopyruvate (3-MP), which is subsequently processed by a mercaptopyruvate sulfurtransferase (MST) to produce pyruvate and H_2S. While pyruvate can fuel mitochondrial respiration, H_2S acts as an antioxidant and signalling molecule. This pathway links sulfur amino acid metabolism, ATP production, and oxidative stress defense, playing a key role in redox homeostasis and enabling *T. cruzi* to adapt to adverse conditions. This study investigates the role of MST in cysteine catabolism in *T. cruzi*. We identified the putative mst gene (*tcmst*) in TriTrypDB (TcCLB.508173.40), encoding a 387-amino acid protein. For biochemical characterization, *tcmst* was cloned into the pET28a(+) vector and heterologously expressed in *Escherichia coli*. Recombinant MST was purified, and preliminary enzymatic assays using 3-MP as substrate confirmed catalytic activity, enabling the determination of kinetic parameters, K_m and V_{max}. To assess MST function, we generated *tcmst* knockout parasites (KO-mst) using CRISPR/Cas9. Proliferation assays revealed distinct phenotypes among MST-deficient clones compared to the parental CAS9 control. Metacyclogenesis assays over seven days showed no significant differences in differentiation capacity. However, oxidative stress assays with hydrogen peroxide demonstrated increased susceptibility in KO-mst clones, supporting the role of MST in tolerance to oxidative imbalance. Additional drug stress experiment using benznidazole showed no significant differences between MST-deficient clones and the CAS9 control. Future work will explore the infectivity of KO-mst parasites in CHO-K1 cells and assess metabolic flux modulation in MST-deficient lines using exometabolomic analyses.
Supported by: FAPESP 2024/06474-9
Keywords: Trypanosoma cruzi;cysteine catabolism;oxidative stress.

HP – 029 - **HETEROLOGOUS EXPRESSION OF *LEISHMANIA MAJOR* GP63 INCREASES *IN VITRO* INFECTION OF *L. TARENTOLAE***

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Leishmaniases are neglected tropical diseases caused by protozoan parasites of the genus ***Leishmania***. The zinc-dependent metalloprotease GP63 is one of the main virulence factors of ***Leishmania*** spp. This surface molecule is a widely associated with cell adhesion, immune modulation, and parasite survival within the host. In non-pathogenic species ***Leishmania tarentolae***, GP63 is expanded in the parasite genoma, but exhibits reduced proteolytic activity due to a reduction in its electrostatic surface potential. To investigate the functional impact of the low enzymatic activity on parasite infectivity, recombinant ***L. tarentolae*** clones were generated to overexpress the homologous GP63, or a heterologous GP63 from ***L. major***. Polyclonal antibodies against GP63 revealed a consistent localization of the protein in intracellular vesicles, cytoplasm, and plasma membrane in all clones. Infection assays using murine peritoneal macrophages reported higher infection rates, and increased amastigote burden in clones expressing ***L. major*** GP63 compared to the wild-type strain. Adhesion assays using Aag2 insect cells also demonstrated enhanced cellular interaction in the heterologous clones. These findings suggest that the reduced enzymatic activity of ***L. tarentolae*** GP63 may be related to its lower infectivity, reinforcing the functional role of GP63 in parasite-host interaction. Furthermore, these results highlight the potential of *L. tarentolae* as a safe and versatile experimental model for functional studies about *Leishmania* sp.
Supported by: Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro: E-26/210.976/2024; Coordenação de Aperfeiçoamento de Pessoal do Nivel Superior: Financial code 001
Keywords: metallopeptidase;immunolocalization;adhesion.

HP – 030 - **Are m6A modifications new regulators of host immunity in *Leishmania* infection?**

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Leishmaniasis is a group of neglected tropical diseases caused by protozoa of the *Leishmania* genus. During its life cycle, the parasite survives as intracellular amastigotes within macrophages in the vertebrate host. At this phase , *Leishmania* faces host immune defenses and has evolved immune evasion strategies, including modulation of host gene expression and metabolic reprogramming, to survive. The N6-methyladenosine (m6A) RNA modification has emerged as a key player in gene expression and immune response during pathogen infection. m6A is regulated by the methyltransferases METTL3, METTL14, WTAP, demethylases FTO, ALKBH5, and binding proteins YTHDF1-3, YTHDC1-2, affecting all life cycle of mRNA and impacting gene expression. Given *Leishmania's* immunomodulatory capacity and the regulatory potential of m6A, we investigated how *L. amazonensis* infection affects m6A levels and its regulatory machinery in macrophages. RNA-seq public data from patients diagnosed with cutaneous and visceral leishmaniasis showed that METTL3 was upregulated and ALKBH5 downregulated post-infection, a result that was validated in infected *versus* non-infected macrophages by RT-qPCR, Western blotting, and immunofluorescence. Global m6A levels, quantified by dot blot, exhibited dynamic and inversely regulated patterns between infected and control conditions over time. Furthermore, direct RNA sequencing identified ~13,500 and ~21,000 m6A-modified transcripts in control and infected macrophages, respectively, including immune-related genes such as *STAT1*, *STAT2*, and *CCL2*, all exclusively detected in infected samples. Ongoing studies aim to clarify the role of m6A, but these findings suggest that m6A plays a significant role in modulating the macrophage transcriptome in response to *Leishmania* infection.

Supported by: 2025/06837-7; 2022/03075-0
Keywords: Leishmania;post-transcriptional modification;m6A.

HP – 031 - **Impact of chronic Leishmania amazonensis infection on hepatic lipid metabolism in C57BL/6 mice**

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Leishmaniasis, a neglected tropical disease caused by *Leishmania* protozoa, depends on the parasite's ability to modulate host lipid metabolism for survival, as it lacks complete lipid biosynthesis pathways. While a great deal is known about acute metabolic changes, the impact of long-term infection is less clear. This study investigated how chronic systemic infection by *Leishmania amazonensis* alters hepatic lipid metabolism in C57BL/6 mice. After a nine-month infection, livers were analyzed by thin-layer chromatography (TLC), colorimetric assays, and Western blotting to assess lipid profiles and the activation state of lipogenic pathways. Infected animals showed increased TAG levels ($p=0.004$), confirmed by TLC ($p=0.02$). Also elevated were 1,2-diacylglycerol ($p<0.01$), free cholesterol ($p=0.02$), and free fatty acids ($p=0.02$). Conversely, monoacylglycerols and oxysterols were reduced ($p=0.02$ each). The phospholipid profile was also significantly altered, showing increased phosphatidic acid, phosphatidylethanolamine ($p=0.02$ each), phosphatidylinositol ($p=0.004$), and sphingomyelin ($p=0.004$), while levels of phosphatidylcholine, lysophosphatidylcholine, and phosphatidylserine were unchanged. Causally, this pro-lipogenic phenotype was linked to reduced inhibitory phosphorylation of both AMP-activated protein kinase (AMPK) and its target, Acetyl-CoA carboxylase (ACC) ($p=0.04$ each), indicating clear activation of the de novo lipogenesis pathway. Our findings demonstrate that chronic *Leishmania amazonensis* infection promotes a profound reprogramming of host hepatic lipid metabolism. By suppressing the AMPK signaling pathway, the parasite activates ACC to drive lipogenesis, likely creating a lipid-rich environment that sustains its long-term persistence. This metabolic manipulation represents a key host-parasite interaction and identifies the lipogenic pathway as a potential therapeutic target. Future work will employ GC-MS to define the specific fatty acids involved.

Supported by: FAPERJ; E-26/203.924/2024
Keywords: Leishmania amazonensis;Host-parasite interaction;Lipid metabolism.

HP – 032 - **PROFILING OF MACROPHAGE MICRORNAS IN RESPONSE TO *T. gondii* AND *N. caninum* INFECTION**

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MicroRNAs (miRNAs) are small endogenous non-coding RNA molecules that regulate post-transcriptional gene expression and are conserved across all eukaryotic species. Recently, their regulatory roles in various biological processes have been extensively studied, highlighting their importance in cellular regulation and their potential as disease biomarkers and therapeutic agents. In this study, we investigated the differential expression profiles of miRNAs during the infection of bone marrow derived macrophages (BMDMs) by *Toxoplasma gondii* or *Neospora caninum*, compared to non-infected cells, aiming to identify miRNAs potentially involved in the host response. MiRNA sequencing (miRNome) was performed to analyze the miRNA expression profiles during infection by these pathogens, allowing the identification, quantification, and comparison of miRNA expression among the groups. This differential expression analysis revealed miRNAs significantly regulated in response to the infections, including a subset of miRNAs highly expressed in the infected cells. We identified differentially expressed miRNAs in both *T. gondii* and *N. caninum* infections, or miRNAs that were differentially expressed in each infection. We chose some of the differentially expressed miRNAs to undergo functional characterization by genomic deletion using CRISPR/Cas9 technology. Our results indicate that those targets are involved in the modulation of immune-related pathways in response to intracellular pathogens. This study highlights the potential role of miRNAs in modulating innate immunity and opens new perspectives for exploring their interactions in the host-pathogen response.
Supported by: FAPEMIG, CAPES, CNPq.
Keywords: microRNAs;Toxoplasma gondii;Neospora caninum.

HP – 033 - **Retinal microvascular alterations associated with cell infiltration and scarring in acquired Toxoplasma gondii infection in C57BL/6 mice**

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1; DE CAMPOS, V.S.1; ZHONG, T.2; CONNOLLY, A.2; RODRIGUES, M.C.P.1; CASCABULHO, C.1; GONZAGA, B.M.D.S.1; CASTRO, L.L.3; ALBA, D.3; PEREZ, V.L.4; GARZONI, L.R.1; CALAZA, K.D.C.5; ADESSE, D.1. 1. INSTITUTO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRAZIL; 2. PRINCETON NEUROSCIENCE INSTITUTE, PRINCETON - UNITED STATES OF AMERICA; 3. BASCOM PALMER EYE INSTITUTE, UNIVERSITY OF MIAMI, MIAMI, FLORIDA - UNITED STATES OF AMERICA; 4. BASCOM PALMER EYE INSTITUTE, MIAMI - UNITED STATES OF AMERICA; 5. UNIVERSIDADE FEDERAL FLUMINENSE, NITERÓI - RJ - BRAZIL.

Ocular Toxoplasmosis (OT) is a major cause of infectious uveitis, leading to retinal inflammation and vascular pathology. We investigated retinal microvascular alterations, immune cell recruitment, and tissue remodeling in a murine model of ocular toxoplasmosis. Adult female C57BL/6 mice were infected intragastrically with two ME49 *T. gondii* cysts and evaluated at 10, 20, and 30 dpi through clinical, molecular, vascular, and histological analyses. Infected mice showed weight loss and reduced food intake starting 10 dpi. Tachyzoites were detected at 10 dpi, and bradyzoites at 20 and 30 dpi. Pro-inflammatory cytokines increased at 10 dpi, anti-inflammatory were increased at 20 dpi. Retinal blood flow and capillary density increased at 10 dpi, followed by vessel length and area remodeling at 20 and 30 dpi. Collagen IV expression rose at 20 and 30 dpi. Endothelial dysfunction appeared at 20 dpi, with reduced vasodilation and abnormal ZO-1, claudin-5, occludin, and VE-cadherin mRNA and protein levels. Leukocyte-endothelial rolling and adhesion increased at 20 and 30 dpi. Retinas were dissociated and analyzed by flow cytometry and we observed $CD4^+$ T helpers, $CD8^+$ cytotoxic T, γδ T cells, and myeloid cells infiltrate at 20 and 30 dpi; NKT cells at 20 dpi; NK cells at 10, 20, and 30 dpi. Glial, microglial and endothelial activation was increased at 20 and 30 dpi. $TUNEL^+$ cell death in the GCL and INL thinning were observed at 20 and 30 dpi. We conclude that *T. gondii* infection induces retinal microvascular alterations, immune cell infiltration, glial activation, and neurodegeneration, leading to neuronal damage in OT.
Supported by: This study was funded by Fiocruz, CNPq, FAPERJ, the INOVA programs, and a Federal Deputy Fund (Marcelo Freixo), through multiple institutional grants, fellowships, and thematic project awards to DA, KCC, and VPPBM.
Keywords: Ocular toxoplasmosis;Toxoplasma gondii;angiogenesis.

HP – 034 - **Selective Anti-*Toxoplasma gondii* Hits from the MMV Global Health Priority Box**

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The clinical management of toxoplasmosis is hindered by drug toxicity and a lack of therapeutic options effective against chronic infection. In line with drug repositioning strategies, a screening of 240 compounds from the Medicines for Malaria Venture (MMV) Global Health Priority Box (GHPB) library was conducted. This library is divided into three subsets: compounds with known activity against disease vectors (VEC), drugs effective against resistant malaria (MB2), and compounds targeting neglected zoonotic diseases (ZND). Screening was performed at a fixed concentration of 1 μM for 72 hours. Compounds were considered active if they inhibited *Toxoplasma gondii* growth by ≥ 80%. Thirty active compounds were identified: 6 from VEC, 10 from MB2, and 14 from ZND. These hits were further characterized by determining their EC_{50} in *T. gondii* tachyzoites, CC_{50} in human foreskin fibroblasts (HFF), and selectivity index (SI). EC_{50} values ranged from 0.01 to 0.90 μM (comparable to pyrimethamine, EC_{50} = 0.381 μM), while CC_{50} values varied from 0.38 μM to > 50 μM. SI values ranged from < 1 to > 755.6. ADMET predictions for the 30 compounds revealed favorable pharmacokinetic profiles for most. Although MMV1794211 (SI > 1,000) was predicted to cross the blood-brain barrier, it was flagged for potential safety concerns. In contrast, MMV674132 (SI > 265), MMV1267536 (SI = 154), MMV006430 (SI > 167) and MMV689404 (SI > 103) combined promising efficacy with more favorable in silico safety profiles, warranting further investigation.

Supported by: FAPESP 2018/18954-4 and 2023/18327-8
Keywords: Drug discovery;Toxoplasma gondii;Medicines for Malaria Venture.

HP – 035 - **TcPAQR4, a novel Trypanosoma cruzi receptor as a target for the treatment of Chagas disease**

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Trypanosoma cruzi, the causative agent of Chagas disease, represents a major public health concern in many countries, including Brazil. Our group recently identified TcPAQR, a *T. cruzi* homolog of the human Progestin and AdipoQ Receptors (hPAQRs), as a putative receptor for platelet-activating factor (PAF) and lyso-phosphatidylcholine (LPC). These receptors belong to the PAQR family, characterized by seven transmembrane domains (7TM). Throughout its complex life cycle, *T. cruzi* undergoes several morphological transitions between epimastigotes, trypomastigotes, and amastigotes, both in its insect vectors and mammalian hosts. Our findings indicate that TcPAQR plays a key role in the differentiation of epimastigotes into trypomastigotes in vitro. Assays with TcPAQR knockdown parasites showed impaired responsiveness to PAF and LPC, confirming the receptor's involvement in signal-mediated differentiation. In the current study, we are conducting molecular dynamics (MD) simulations of TcPAQR embedded in a *T. cruzi* membrane in five configurations: (1) ligand-free, (2) bound to PAF, (3) bound to LPC, (4) bound to WEB 2086 (a known PAQR inhibitor), and (5) bound to the most promising *in vitro* candidate. These simulations aim to elucidate the molecular mechanisms underlying TcPAQR activation and inhibition. Our MD protocol, developed in-house, is currently being validated using the ligand-free system. This study aims to elucidate how LPC, PAF, and WEB 2086 interact with TcPAQR. Previously, we modeled TcPAQR and performed a virtual screening of over 500,000 compounds from a private chemical library. Eighteen candidate inhibitors were selected for further analysis based on molecular dynamics and ligand-receptor interaction studies. Molecules that meet the criteria of *in vitro* assays will support the validation of these compounds as promising drug candidates for the treatment of Chagas disease.

Supported by: CNPq, CAPES, FAPERJ, INCTEM
Keywords: Trypanosoma cruzi;Drug Design;Computational Bbiology.

HP – 036 - **Evaluation of Anti-Toxoplasma gondii Activity and Cytotoxicity of Five Repurposed Compounds with Favorable Pharmacokinetic and Toxicological Profiles**

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Introduction: Toxoplasmosis is a zoonotic disease caused by *Toxoplasma gondii*, which can lead to clinical manifestations. Pharmacological intervention is essential following diagnosis, with pyrimethamine being the standard treatment. However, its clinical use is limited by significant adverse effects, highlighting the need for safer and more effective therapeutic alternatives. Drug repurposing emerges as a promising strategy, allowing the reuse of compounds with established safety profiles and commercial availability. **Objective:** To identify FDA-approved drugs with favorable pharmacokinetic and toxicological properties and selective activity against *T. gondii*. **Methods:** A virtual screening was performed using the SwissADME and OSIRIS platforms to select FDA-approved compounds with predicted high gastrointestinal absorption, ability to cross the blood-brain barrier, and no toxicity. The screening was conducted using libraries provided by the Medicines for Malaria Venture (MMV). Selected compounds were subsequently evaluated in vitro to determine their 50% Effective Concentration (EC_{50}) against *T. gondii* tachyzoites and 50% Cytotoxic Concentration (CC_{50}) against human foreskin fibroblasts (HFF). Selectivity was assessed by calculating the Selectivity Index (SI), defined as the ratio between CC_{50} and EC_{50} values. **Results:** The virtual screening of 560 compounds led to the selection of five drugs: clemastine, loratadine, papaverine, pilocarpine, and terconazole. These compounds showed SI values ranging from 2.20 to >42.37. Pyrimethamine, used as a reference drug, exhibited an SI >135. **Conclusions:** Although pyrimethamine showed higher SI, safer alternatives must be explored. The five drugs identified demonstrated relevant in vitro selectivity and represent promising candidates for toxoplasmosis treatment. Future steps include assessing in vivo efficacy in a murine model of chronic toxoplasmosis and elucidating the anti-*T. gondii* mechanisms of action of these compounds.

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Keywords: Toxoplasma gondii;Drug repurposing;Parasitic diseases.

HP – 037 - **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*. In its life cycle, when feeding on an infected host, the triatomine acquires trypomastigote forms. In the anterior midgut, epimastigote forms emerge, which interact with the intestinal wall, invade the hemolymph, and penetrate the hemocytes, where they differentiate. Upon the rupture of these cells, new forms are released. Due to the absence of a complete lipid metabolism, the parasites must acquire lipids from the invertebrate host in order to survive and proliferate. Infection can cause various complications for the insect, including death. Therefore, *T. rangeli* may act as a potential biological control agent for Chagas disease by reducing triatomine populations.

This study aimed to evaluate the impact of *T. rangeli* infection on lipid transport during oogenesis in *R. prolixus*. To this end, 24 hours after feeding, 10^4 forms of the parasite were injected. Three days later, the insects were dissected and their ovaries analyzed by thin-layer chromatography. In a separate experiment, tritiated palmitate was injected to investigate the influence of infection on lipid dynamics. Assays such as electrophoresis were used to assess lipophorin and vitellogenin levels in the hemolymph.

The results revealed alterations in lipid levels in infected insects, with reduced triacylglycerol (TAG), fatty acids (FA), and diacylglycerol (DAG) in the ovaries. Tritiated palmitate indicated impaired lipid transport to the ovaries. A significant decrease in vitellogenin levels was also observed in the hemolymph. These data suggest that *T. rangeli* impairs lipid mobilization and storage, affecting egg production and reducing the reproductive rate of the insects.

Supported by: CAPES
Keywords: Rhodnius prolixus;Trypanosoma rangeli;Lipid.

HP – 038 - **Evaluation of the Establishment and Maintenance of Dormancy in Trypanosoma cruzi**

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Dormancy has been recognized as playing a crucial role in *Trypanosoma cruzi* survival, enabling its persistence in adverse conditions. Dormancy has been associated with a reduction in metabolic activity and cell proliferation.This event has a significant impact on both the pathogenesis of Chagas disease and the development of effective therapies. Dormant *T. cruzi* cells are resistant to treatments, such as benznidazole and nifurtimox, which are commonly used during the acute phase of the disease. Understanding the mechanisms involved in the induction and maintenance of dormancy is essential for developing therapeutics that may eradicate the parasites. This study aims to identify markers related to dormant parasites. Dormant cells were recognized by a decreased 24-hour incorporation of 5-Ethynyl-2'-deoxyuridine (EdU), in parallel with immunofluorescence staining for various parasite antigens. Our results indicated that quiescent parasites exhibit a notable reduction in their nucleolus, implying a decrease in ribosomal synthesis and in translational activity. This decrease was unrelated to phosphorylation of the eukaryotic translation initiation factor 2, which triggers stress responses with protein synthesis inhibition, as mutant parasites lacking the phosphorylation site of this factor did not exhibit an increase in dormant parasites. Furthermore, spontaneous dormancy is strain-dependent; parasites of the Y strain show a higher incidence of spontaneous dormancy compared to those of the Dm28c strain. Highly virulent parasites, obtained after 20 passages through mice, had a lower incidence of dormancy than parasites cycled in mammalian cells in vitro. Finally, we observed that infected cells in the presence of high glucose show a decreased number of dormant parasites. Overall, we conclude that features related to parasite environmental fitness affect the presence and entry in the quiescence.

Supported by: CAPES - 88887.821068/2023-00

Keywords: Trypanosoma cruzi;Dormancy;Molecular mechanisms.

HP – 039 - **Action of glycosome blockers on *in vitro* infections by *Leishmania (Leishmania) amazonensis* and *Leishmania (Leishmania) infantum*.**

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Leishmaniasis are a group of neglected diseases with an annual incidence of approximately 1 million cases for the cutaneous form and 30,000 for the visceral form. Both forms are endemic in Brazil, representing a significant public health issue. Current treatments are often unsatisfactory due to their toxicity, variable efficacy, or the emergence of relapses. Glycosomes are organelles present in Trypanosomatidae protozoans that house most of the enzymes of the glycolysis and other metabolic pathways, which are produced in the cytoplasm and transported to the glycosome with the help of PEX proteins. The PEX5-PEX14 interaction is essential for this transport. Recent studies have developed a 3D pharmacophore model that mimics the binding of PEX5 to PEX14. Compounds developed using this model blocked PEX14-PEX5 interaction and were toxic to *Trypanosoma brucei* and *T. cruzi*, with low toxicity to mammalian cells. Considering the limitations of current therapies and the metabolic importance of glycosomes, we evaluated 19 synthetic PEX14–PEX5 inhibitors. Four compounds were selected for their efficacy and favorable *in silico* ADME parameters: DOL630, TF243, TF254, and TF257. All compounds reduced promastigotes viability after 72 hours of incubation, with EC50 values ranging from 5.2 ± 2.1 μM to 60.1 ± 14.5 μM for *L. (L.) infantum* and from 5.6 ± 0.7 μM to 15.4 ± 1.7 μM for *L. (L.) amazonensis*. Toxicity in murine macrophages (RAW 264.7 cells) and human macrophages (THP1 cells) was relatively low (CC50: 8.4-51.8 μM). In infected RAW 264.7 cells all compounds showed leishmanicidal activity against intracellular amastigotes (EC50: 1.4 ± 0.1–3.3 ± 0.4 μM), with selectivity indices from 5.2 to 22.3, indicating promising antiparasitic potential with limited host toxicity.

Supported by: CNPq

Keywords: PEX14–PEX5 inhibitors;Glycosome;Drugs.

HP – 040 - **Dynamics of transcription and subcellular localizations of Trypanosoma rangeli proteins in the context of Rhodnius prolixus infection**

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Trypanosoma rangeli is a non-pathogenic hemoflagellate protozoan that infects mammalian hosts and hemipteran vectors. It coexists with *Trypanosoma cruzi*, the causative agent of Chagas disease, across Central and South America. Unlike *T. cruzi, T. rangeli* epimastigotes (EPI) can evade the gut of its vectors, replicate in the hemolymph, and migrate to the salivary glands, where they differentiate into infective metacyclic trypomastigotes (MT) that are inoculated into the mammalian bloodstream during probing. This study employed RNA-seq to investigate the life cycle changes that *T. rangeli* undergoes during its infection of *Rhodnius prolixus*, focusing on the hemolymph and salivary glands of the infected vector to analyze gene expression and localization throughout parasite development. Sequences generated by Illumina HiSeqX were assembled with Trinity, quantified with Salmon, and analyzed for differential expression using DESeq2. Transcripts of EPI and MT showing FDR <0.01 and log2FC >1.5 were considered upregulated. Subcellular localization was predicted using WoLF PSORT, and statistical differences between EPI and MT were assessed by Fisher's Exact Test (FDR <0.05). Up-regulation of transcription of genes related to the extracellular space (82), peroxisome (8), ER (11), plasma membrane (15), mitochondrion (15), nucleus (22), and cytosol (27) was observed for infective metacyclic trypomastigotes. These genes are related to biologically relevant aspects such as host interaction, immune modulation, and metabolism. For EPI, difference was exclusively observed for extracellular location (27), among which, upregulation was observed for cellular functions related to cell division, such as protein folding/degradation, host interaction, nucleic acid repair, and cellular structure. The identification of these upregulated transcripts correlates with biological mechanisms related to the parasite infectivity, replication, and pathogenicity to the invertebrate hosts.

Supported by: CAPES/STINT 23038.003782/2014–11
Keywords: RNA-seq;Trypanosomatids;Vector.

HP – 041 - **What are the consequences of mitogenic stimulation for Trypanosoma cruzi, the etiological agent of Chagas disease?**

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Trypanosoma cruzi is the etiological agent of Chagas disease (CD), a neglected illness that primarily affects low-income populations in the Americas. The CD treatment is challenging due to the high toxicity of the current drugs available and the persistence of dormant parasite forms. Therefore, the investigation of new therapeutic strategies is essential. Based on seemingly paradoxical recent findings in cancer cells, we hypothesize that cells with higher replication rates experience more pronounced DNA damage. Thus, mitogenic stimulation could be a strategy to activate dormant parasite forms, making them susceptible to combination treatments that inhibit DNA damage response, potentially leading to clearance. To test this hypothesis, we chose LB-100, a competitive inhibitor of the protein phosphatase 2A (PP2A), as a potential candidate. We used three concentrations of LB-100 on epimastigote forms of the T. cruzi CL Brener strain, and no significant phenotypic changes were observed, based on growth curves, cell viability (MTT assay), and DNA content analysis by flow cytometry. Apparently, PP2A is expressed only in T. cruzi amastigote forms, which explain our results. The evaluation of the LB-100 effect on the intracellular forms of T. cruzi is being carried out using AC16 human cardiomyocytes. Both the intracellular replication rate of parasites and the LB-100 toxicity to human cells are being investigated. Preliminary results show a reduction in the number of T. cruzi amastigotes per infected cell starting at 72 h post-treatment. Next steps include bioinformatic comparisons of PP2A in T. cruzi and humans, and investigation of DNA damage response in T. cruzi cells. These findings suggest that even without visible increases in parasite load, LB-100 may exert significant cellular effects on T. cruzi amastigotes, like those seen in cancer models, supporting its potential as a future therapeutic strategy.

Supported by: FAPESP - 2024/07821-4
Keywords: DNA damage response;mitogenic activation;LB-100.

HP – 042 - **Molecular Investigation of Serine Peptidase Inhibitor 2 Variants from Amazon Region Strains of *Trypanosoma cruzi***

SANTANA, C.M.[1]; SPERANCA, M.A.[1]; LAPORTA, G.Z.[2]; ABREU, W.U.[1]; JORDÃO, F.T.[1]; BRILHANTE, A.F.[3]; MADEIRA, F.P.[3]; C.ILACQUA, R.[4].
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Chagas disease (CD) is a zoonosis caused by the protozoan *Trypanosoma cruzi*, belonging to the Kinetoplastida Order, Trypanosomatidae family, Schyzotripanum subgenus and *Trypanosoma* genus, being the third most common cause of parasitic infection in the world and the most important in Latin America. *T. cruzi* infection can occur through vector transmission by triatomines, arthropods of the Reduviidae family; congenital disease and by ingestion of infected triatomines. After the initial infection, the acute phase with severe symptoms may occur, but CD commonly progresses to the chronic or indeterminate phase, characterized by the absence of clinical signs and symptoms of cardiac or digestive involvement, which may be related to different genetic populations of *T. cruzi.* According to the most recent classification, there are six genetic populations of *T. cruzi*, distributed according to the region and habits of the transmitting triatomines. This knowledge 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, the detection of parasite nucleic acids can be performed by real-time PCR or qualitative PCR (low sensitivity in clinical samples) with markers that do not allow the identification of DTUs. Therefore, further studies are needed to obtain molecular and serological markers for the diagnosis of CD. The objective of this study was to investigate the role of the *T. cruzi* serine peptidase 2 inhibitor (TcISP2) in the diagnosis of CD. The evaluation of the gene encoding TcISP2 as a marker for molecular diagnosis was carried out on samples from the project “Cohort of Zoonotic Landscapes Under the Effect of Deforestation and Land Use Change in the Amazon,” collected in the city of Cruzeiro do Sul, Acre.

Supported by: Fundação de Amparo a Pesquisa - FAPESP
Keywords: Chagas disease;T; cruzi;ISP2.

HP – 043 - ***Leishmania infantum* E-NTPDase2: analysis of the protein interactome in overexpressing mutants**

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Ecto-nucleoside triphosphate diphosphohydrolases (E-NTPDases) hydrolyze extracellular tri- and diphosphate nucleotides and occur in many organisms. *Leishmania infantum*, the causative agent of visceral leishmaniasis in the Americas, encodes two isoforms—LiNTPDase1 and LiNTPDase2—with evidence suggesting a predominant role of LiNTPDase2 in parasite–host interactions. This study aimed to biologically characterize LiNTPDase2 by investigating its interactions with other proteins from the parasite. Co-immunoprecipitation assays were performed using protein extracts from *L. infantum* JPCM5 promastigotes expressing LiNTPDase2–eGFP–H6 and eGFP (control), followed by immunocapture with anti-GFP antibodies and protein A/G magnetic beads. Eluted proteins were analyzed by LC–MS/MS, and data were processed in PEAKS Studio with a 0.1% FDR threshold. Analyses revealed several potential partners, including hypothetical proteins. Notably, LiNTPDase1 was identified, suggesting a functional interaction between isoforms. Ribosomal proteins, factors related to ribosome biogenesis, and mRNA-binding proteins were recurrent, indicating a possible role in translational regulation. Other prominent targets included proteins related to transport and metabolism, such as an aspartyl-tRNA synthetase and an amino acid permease. Additionally, the identification of the dynein heavy chain, a motor protein, suggests that LiNTPDase2 may have a broad subcellular distribution. We are currently deepening our analysis of these interactions through in silico and in vitro approaches to validate the findings. The study of LiNTPDase2 and its partners may reveal novel targets and therapeutic strategies against visceral leishmaniasis, contributing to parasite biology knowledge and disease control. FAPEMIG, CNPq, BIOAGRO, UFV, and CAPES supported this research.

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Keywords: Leishmania infantum;E-NTPDase2;interactome.

HP – 044 - **LAMP-Based Assay for Visceral Leishmaniasis**

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Visceral leishmaniasis (VL) is one of the foremost neglected tropical diseases, affecting thousands of people worldwide, particularly in Brazil, where it is predominantly caused by *Leishmania infantum*. It is a zoonosis that infects dogs, the principal urban reservoirs of the parasite. The diagnosis comprises parasitological, serological, and molecular techniques; however, these approaches require sophisticated laboratory infrastructure and specialized personnel, which limits their application in emergency care units. Consequently, the development of rapid, simple and effective tests is essential for early diagnosis, prompt initiation of treatment and ongoing monitoring of VL. Loop-mediated isothermal amplification (LAMP) has emerged as a promising alternative, enabling DNA amplification at a constant temperature, thus dispensing with the thermocyclers required for conventional PCR. Due to its high sensitivity, specificity and simplicity, LAMP has proved a valuable tool for diagnosing infectious diseases in resource-limited settings. When combined with visual read-out methods, such as lateral flow strips (LFS), it can yield an affordable test for field or point-of-care use. This study aimed to standardize LAMP reactions with specific primers described in the literature for the detection of *L. infantum* and to adapt these primers with markers such as biotin and FAM (6-carboxyfluorescein). Reactions were optimized for temperature (65 °C), duration (40 min), dNTP concentration (1.4 mM) and $MgSO_4$ concentration (8 mM), and are currently being assessed for specificity against other Leishmania and Trypanosoma species. The proposal also encompasses the development of strips containing particles conjugated to anti-FAM antibodies and a test line with immobilized streptavidin, with the objective of producing a simple, rapid and effective assay for VL diagnosis

Supported by: Capes, FAPEMIG e CNPq
Keywords: visceral leishmaniasis;LAMP;diagnosis.

HP – 045 - **Long-term IL-4 stimuli polarize macrophages towards M2a phenotype but fails to increase susceptibility to Leishmania braziliensis**

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Leishmaniasis is caused by protozoan parasites transmitted by sand-flies. Host Th1 responses promote the killing of intracellular parasites while Th2 responses lead to susceptibility and parasite proliferation. Macrophages stimulated by Interferon-γ (IFN-γ) are polarized towards the M1 phenotype that can eliminate parasites primarily by the production of reactive nitrogen species (RNS) and/or reactive oxygen species (ROS), while macrophages stimulated by IL-4 shift their polarization towards the M2a phenotype that favors wound healing and inhibits pathways responsible for the production of RNS. We aimed to evaluate long-term IL-4 stimulation (LT-IL4) effects on macrophage polarization and susceptibility to *L. braziliensis*. LT-IL4 reduced RNS production post-IFN-γ stimulus without affecting ROS, while increasing arginase activity and urea. Surface marker analysis revealed that LT-IL4 reduced the number of cells expressing CD86, MHCII, TLR2, and PDL1, while increasing both the number of CD206-expressing cells and the intensity of CD206 expression. When the LT-IL4 cells were challenged with IFN-γ, they showed no difference in expression of markers relative to control group challenged with IFN-γ. We observed that LT-IL4 cells are resistant to infection with *L. braziliensis* promastigote infection, similar to control. When submitted to infection with resistant *Leishmania braziliensis* amastigotes, LT-IL4 cells were susceptible, similar to control; subsequent stimulation with IFN was able to repolarize cells towards M1 phenotype and promote killing of intracellular parasites in both groups. These results suggest that LT-IL4 can generate a stronger M2a phenotype and inhibit RNS; however, cells are still plastic enough to revert surface marker expression and maintain their parasite killing function.

Supported by: CAPES
Keywords: Alternative activation;Macrophage plasticity;Reactive nitrogen species.

HP – 046 - **The potential of coxibs, a non-steroidal anti-inflammatory class of drugs, as an anti-*Trypanosoma cruzi* candidate, and its interference on energy metabolism.**

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Chagas disease affects 6-7 million people and only two drugs are available for the treatment of the disease, nifurtimox and benznidazole. Both drugs present limitations regarding efficacy and tolerance. In this context, there is an urgent need to know in depth the metabolism of this protozoan to identify and validate new therapeutic targets. It is known that proline (l-Pro) is important for several biological processes in *T. cruzi*. The l-Pro degradation pathway consists of two mitochondrial enzymes: proline dehydrogenase [PRODH EC: 1.5.5.2] and Δ^1-pyrroline-5-carboxylate dehydrogenase [P5CDH EC: 1.2.1.88]. PRODH is responsible for the oxidation of l-Pro to pyrroline-5-carboxylate, which is converted by a non-enzymatic reaction into glutamate-1-semialdehyde (GSA) and P5CDH oxidizes GSA to glutamate. Recent studies have evidenced that celecoxib, a non-steroidal anti-inflammatory drug, is capable of inhibiting human PRODH from tumor cells. In this context, the potential trypanocide effect of six coxibs drugs was investigated, and the mitochondrial metabolism was assessed. Molecular docking analyses showed that celecoxib and valdecoxib generated the best poses and scores with ASP fitness 42.61 and 32.54, respectively. To assess the effect of coxibs on *T. cruzi*, the proliferation of epimastigotes was measured at different compounds concentrations (1.56-100 µM) allowing the IC_{50} determination. The obtained results demonstrated that celecoxib and valdecoxib resulted in a proliferation reduction of ~90% at 50 µM, equivalent to an IC_{50} of 31.3 µM and 35.5 µM respectively. To determine whether celecoxib affects mitochondrial metabolism, epimastigotes were incubated at the IC_{50} concentration for 5 days, and the cellular oxygen consumption was measured by high-resolution respirometry. Celecoxib was able to inhibit the free routine activity by 44.6% and maximum electron transfer capacity by 68.5% when compared with non-treated control. Celecoxib also demonstrated a CC_{50} >200 µM on CHO-K_1, evaluated by MTT assay. To assess the effect on cell-released trypomastigote forms, celecoxib was tested at 1.56-50 µM and showed an IC_{50} of 14.73 µM and IS >10. Taken together, our results showed that celecoxib has an anti-*T. cruzi* activity. Further analyses will be performed to identify the inhibitory effect of celecoxib on *Tc*PRODH.
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Keywords: Chagas disease;Drug repositioning;Proline metabolism.

HP – 047 - **Developing Host Directed Therapies for Chagas Disease**

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Chronic infection with the protozoal parasite *Trypanosoma cruzi* causes persistent inflammation and subsequent clinical symptoms known as Chagas Disease (CD). CD is the leading cause of non-ischemic cardiomyopathy in Latin America, and is an emerging threat in the US. The inflammatory infiltrate into tissues of patients with CD includes macrophages, neutrophils, and CD4+ and CD8+ T cells. These immune cells produce proinflammatory cytokines including IFNγ, TNFα and IL-6, which exacerbate tissue damage, leading to organ dysfunction. Regulatory immune cells, including myeloid derived suppressor cells (MDSCs), regulatory DCs (DCs) and regulatory T cells (Tregs), proliferate and are recruited to tissues to ameliorate inflammation. However, regulatory immune cells induced by *T. cruzi* infection have impaired immunosuppressive functions and are thus insufficient to completely resolve inflammation. We have developed an experimental mouse model that partially replicates human CD, with increased cardiac IFNγ, TNFα and IL-6 and CD8+ cells, as well as increased regulatory DCs and MDSCs in the spleen. However, Ly6G+ stained cells in the heart are reduced, concurrent with no increase in expression of the chemokine CCL5 or chemokine receptors CXCR4 and CCR5 in *T. cruzi* infected mice compared to naïve controls. In contrast to *T. cruzi* infection, helminth parasites induce anti-inflammatory immune responses as an immune evasion mechanism to persist within hosts. Here we show that a recombinant protein derived from hookworm excretory/secretory products, designated AIP-1, can induce increased percentage of Ly6G+ stained cells in the hearts of mice chronically infected with *T. cruzi*, concurrent with increased CXCR4, CCR5, and S100A8 and S100A9 expression. Importantly, in treated mice the proinflammatory cytokines IFNγ, TNFα and IL-6 as well as CD8+ cells were reduced in the heart. Thus hookworm derived proteins could be a novel host directed anti-inflammatory therapy to treat CD.
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Keywords: Trypanosoma cruzi;Inflammation;therapeutic.

HP – 048 - **Biochemical and Structural Characterization of Acetate:Succinate CoA-Transferase and Effects of Heterozygous Knockout on glucose and amino acids metabolization: insights from exometabolomics analysis on energy metabolism.**

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Trypanosoma cruzi, the etiological agent of Chagas disease, exhibits unique metabolic features that distinguish it from human cells. Among them, there is an enzymatic cycle composed of acetate:succinate CoA-transferase (ASCT) and succinyl-CoA synthetase (SCS), which is potentially involved in ATP synthesis through substrate-level phosphorylation (SLPHOS) from acetyl-CoA and succinate. This cycle produces ATP in a 1:1 stoichiometry with acetate, which may be reused for lipid biosynthesis or excreted. In this study, we reconstituted the ASCT/SCS cycle in vitro and performed a biochemical characterization of TcASCT. The enzyme exhibited a K_M of 4.87 ± 0.095 mM for succinate, 0.089 ± 0.005 mM for acetyl-CoA, and 0.031 ± 0.002 mM for *n*-propionyl-CoA, indicating its highest affinity for n-propionyl-CoA. The k_{cat} values were 173 ± 1.68 s^{-1} (succinate), 146 ± 2.44 s^{-1} (acetyl-CoA), and 48.3 ± 0.955 s^{-1} (*n*-propionyl-CoA), demonstrating that succinate is the most efficiently processed substrate despite its lower binding affinity. We found that ASCT follows a classical ping-pong bi-bi reaction mechanism, with acetyl-CoA binding first releasing CoA, and followed by binding of succinate and succinyl-CoA release. Heterozygous knockout strains were generated for all cycle components, including both SCS subunits, resulting in >90% transcript reduction. The ASCT hemi-knockout clones exhibited significantly reduced cell proliferation (~51.7%) and decreased acetate excretion flux from glucose (~40.3% reduction), pyruvate (~83.4% reduction), serine (~89% reduction), and threonine (~52.8% reduction) metabolism, along with reduced glycine excretion from threonine (~36.8% reduction), which indicates ASCT as one of the main routes for acetate production in *T. cruzi*. Additionally, we resolved the ASCT structure at 3.5 Å resolution, revealing a heterotetrameric conformation. Together, our findings highlight the central role of the ASCT/SCS cycle in carbon utilization and suggest that the ASCT/SCS cycle functions as a key metabolic node in *T. cruzi*, connecting amino acid and carbohydrate metabolism to intramitochondrial ATP production by SLPHOS and providing precursors for lipid biosynthesis.
Supported by: FAPESP: 2022/16078-8 and 2021/12938-0
Keywords: Trypanosoma cruzi;Metabolsim;bioenergetics.

HP – 049 - **Localization and functional assessment of MAG1 secreted protein in *Neospora caninum.***

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Dense granule proteins (GRAs) are secreted by apicomplexan protozoa after host cell invasion, playing essential roles in maintaining the parasitophorous vacuole (PV) and modulating the host immune response. MAG1 is a GRA protein released by *Toxoplasma gondii* into the PV during the tachyzoite stage and, in the bradyzoite stage, is associated with the cyst matrix and wall. Its expression in both stages and recognized immunogenicity reinforce its potential as a research target. However, there is no information about its orthologue in *Neospora caninum*. In order to check whether the protein is actually expressed in *N. caninum*, the MAG1 gene was endogenously tagged with three hemagglutinin epitopes, and the protein expression was confirmed by immunofluorescence assay and Western blot analysis. To assess its functional role, the MAG1 gene was deleted using CRISPR/Cas9, and the impact of protein absence was evaluated through an in vitro plaque assay, as well as in vivo infections in mice, where we quantified the parasite load quantification in peritoneal fluid and brain by qPCR, and IL-12p40 measurement in peritoneal fluid and serum by ELISA. Mice infected with the knockout line showed reduced in vitro growth compared with the wild type parasites. Parasite load was similar in mice infected with both lines in the peritoneal fluid (1 dpi), whereas the chronic phase parasite burden in the brain (30 dpi) was lower in mice infected with the knockout strain. IL-12p40 levels were increased in infected animals compared to non-infected controls ($p < 0.05$), with no significant difference between infected groups. These findings suggest that MAG1 may be involved in parasite growth and cerebral persistence, although further studies are required to fully elucidate its role in *N. caninum* infection.
Supported by: FAPEMIG, CAPES, CNPq
Keywords: MAG1;Dense granule protein;Neospora caninum.

HP – 050 - **Physiological and behavioral profile of *Rhodnius prolixus* infected by *Trypanosoma rangeli***

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Triatomines are nocturnal insects that feed on blood throughout all life stages. Energy from their diet is stored as triacylglycerides in the fat body and transported via hemolymph to different organs by a specific carrier, lipophorin (lp). These insects can transmit *Trypanosoma rangeli*, a parasite that is not pathogenic to humans, but can produce different levels of pathogenicity, as well as physiological and behavioral alterations, to its invertebrate hosts. In a previous study, we showed that fifth-instar *Rhodnius prolixus* nymphs infected with *T. rangeli* exhibit higher amounts of glycerides in the hemolymph and fat body, along with increased locomotor activity when compared to uninfected nymphs. Additionally, infected nymphs showed upregulated expression of the *diacylglycerol acyltransferase (dgat*) and *lp* genes, suggesting that *T. rangeli* manipulates the lipid metabolism of its insect host. Based on these results, this study aimed to investigate the role of *dgat* and *lp* genes in *T. rangeli* development by silencing these genes in fifth-instar *R. prolixus* nymphs using RNAi interference (RNAi). Silencing was performed 10 days post-molting, achieving suppression rates of 83,37% (dgat) and 97,01% (lp) at 20 days post-inoculum. Seventeen days post-molting, *T. rangeli* was inoculated into the hemocoel of *lp*-silenced and control nymphs, and parasite growth in hemolymph was analyzed. Ten days post-infection (20 days post-silencing) *lp*-silenced insects showed increased *T. rangeli* amounts compared to controls. However, by 60 days post-silencing, parasite levels were similar between treatments. This result was associated to a reduction in the levels of gene silencing. These findings suggest that lipophorin influences early parasite growth, though further validation with larger sample sizes is underway.

Supported by: Vice Presidência de Pesquisa e Coleções Biológicas - FIOCRUZ; FAPEMIG
Keywords: Lipophorin;DGAT;Vector-parasite interaction.

HP – 051 - **Impact of Mast Cell-Derived DETs on Leishmania amazonensis-Macrophage Interaction**

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Leishmaniasis is a neglected tropical disease caused by protozoa of the genus *Leishmania*, transmitted by sandflies. After being inoculated into the skin, the parasites interact with innate immune cells, among them mast cells and macrophages, the latter being the primary host cell where they survive and multiply. Previous studies have shown that *Leishmania* induces **DNA Extracellular Traps (**DETs) in murine mast cells, rat basophilic leukaemia cells, and the human mast cell line (HMC-1). DETs can immobilize pathogens and influence surrounding immune cells, making them potentially important modulators of infection and host response. These DETs are composed of a DNA scaffold associated with bioactive proteins, which trap the parasites and could also impact bystander cells. This work aims to study the impact of these DETs on macrophages. Initially, DETs were induced with *Leishmania amazonensis* live promastigotes in the HMC-1 cell line. DETs were isolated and quantified by Picogreen assay. We then assessed the toxicity of these DETs for human macrophages derived from peripheral blood mononuclear cells by quantifying lactate dehydrogenase release in the culture supernatants. Our results showed 14% toxicity at 50 ng/mL of DETs, while treatment with 10 ng/mL of DETs had little impact on macrophage viability. Then, we infected human macrophages with *L. amazonensis* promastigotes for 24 h, and treated them with different concentrations of isolated DETs. Our results showed that 10 ng/ml DETs significantly reduced parasite load, percentage of infected cells, and endocytic index after treatment. Further, we assayed DETs endocytosis by human macrophages, and observed that macrophages are capable of internalizing the webs. These findings suggest that DETs not only reduce *Leishmania* infection in human macrophages but are also actively internalized by these cells, which open new perspectives for exploring DETs as potential modulators of the immune response.

Supported by: CAPES
Keywords: Macrophages;DNA Extracelullar Traps;Leishmania amazonensis.

HP – 052 - **Evaluation of trehalose metabolism in *Rhodnius prolixus* infected with *Trypanosoma rangeli***

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Trypanosoma rangeli is a hemoflagellate parasite that induces pathogenic effects in *Rhodnius prolixus.* Previous studies have shown that *T. rangeli* infection alters lipid metabolism and locomotor activity in *R. prolixus*. Trehalose, the most abundant carbohydrate in insect hemolymph, serves as a rapid energy source. In this context, the present study evaluated the impact of *T. rangeli* infection on trehalose reserves in *R. prolixus*. First, we quantified trehalose levels in uninfected fifth-instar nymphs at different time points post-molting (days 0, 5, 10, 15 and 30) and post-feeding (days 0, 5, 10 and 15) using a colorimetric enzymatic kit. Results showed a trehalose peak in the day of ecdysis and in the first days after feeding. Subsequently, we compared trehalose levels in the hemolymph and fat body of *T. rangeli*-infected and uninfected fifth-instar nymphs. The results showed a significant reduction of this carbohydrate in infected insects, in both tissues evaluated. Locomotor activity, measured via actometers over 24 hours, was also compared between infected and uninfected fifth-instar nymphs. Hemolymph trehalose quantification in these insects, showed a negative association between locomotion and trehalose amounts in infected individuals. Finally, RT-qPCR analysis of the trehalose transporter gene (*tret*) showed no differential expression between infected and uninfected groups. In summary, our findings show that *T. rangeli* infection promotes alterations in trehalose contents of *R. prolixus*, suggesting a potential manipulation by the parasite.

Supported by: FAPEMIG
Keywords: Trehalose;Carbohydrate;Vector-parasite interaction.

HP – 053 - ***Leishmania amazonensis* promastigotes are not killed and trigger the formation of DNA extracellular traps (DETs) in human mast cell line**

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Mast cells are components of the innate immune system, and are widely distributed in the skin where they express various pattern recognition receptors (PRRs). *Leishmania* promastigotes are inoculated in the skin by the sandflies, where they may interact with mast cells. Research has shown that when stimulated by *Leishmania*, mast cells can release DNA extracellular traps (DETs) associated with different proteins. Here, we aimed to evaluate, in human mast cell line (HMC-1) stimulated with *Leishmania amazonensis* promastigotes (MOI 5), some of the proteins associated with DETs' DNA. Our findings showed that DETs induced by *Leishmania* are decorated with H3 citrullinated histone, β-hexosaminidase, tryptase and LL-37. Similar to neutrophil elastase in the neutrophils' NET release, we showed that tryptase migrates to the HMC-1 nucleus. We also tested the role of TLR4 in this process by treating HMC-1 cells with a TAK-242 inhibitor before parasite interaction. Our results showed that indeed, DET release was decreased by this treatment. Next, we evaluated the origin of the DNA present in those DETs by PCR. Our results demonstrated that the DETs' DNA scaffold is composed of nuclear and mitochondrial origin. We assayed the toxicity of DETs for the parasites and for human fibroblasts (HFF-1) using the resazurin test, showing that DETs have no toxic effect against fibroblasts at the concentrations and times (2 - 4h) tested. Interestingly, although DETs did not kill promastigotes in our conditions, the parasites are ensnared by the webs. Our results provide novel insights into the process of DET formation and function, induced by *Leishmania amazonensis* promastigotes in HMC-1 cell line.

Supported by: CNPq
Keywords: Mast cell;Leishmaniasis;DNA extracellular Traps.

HP – 054 - **Modulation of Vector Attraction by Trypanosome-Infected Hosts**

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Parasite-induced behavioral manipulation is a multifaceted phenomenon that alters host behavior to favor parasite transmission. A strategy used by parasites with complex life cycles involving multiple hosts is to increase proximity between intermediate and definitive hosts. Our group previously showed that Rhodnius prolixus infected with T. cruzi or T. rangeli display altered locomotor activity and greater predation risk than uninfected conspecifics. Here, we investigated whether T. cruzi or T. rangeli infection alters mammalian hosts to increase their attractiveness to insect vectors. Choice assays conducted in glass experimental arenas revealed that nymphs were not more attracted to T. cruzi-infected mice. In contrast, in assays with T. rangeli-infected mice, nymphs were significantly more attracted, suggesting that this effect is specific to T. rangeli infection. However, in choice assays where insects could interact directly with the vertebrate host, this increased attractiveness did not result in higher predation rates, suggesting a potential adaptive advantage for the parasite, as a larger proportion of insects fed on the hosts and became infected. We then sought to identify which host cues or behaviors might be altered during infection and lead to increased attractiveness. Choice assays recorded with infrared-sensor cameras showed no differences in nocturnal activity between infected and uninfected mice. Odor is one of the cues insects used to locate their hosts, we collected volatile compounds from T. rangeli-infected and uninfected animals using dynamic headspace sampling. Preliminary results revealed alterations in the odor profile of infected animals, suggesting that chemical compounds present in the odor of T. rangeli-infected hosts may act as cues modulating vector host-seeking behavior. This study provides new insights into T. rangeli–mammal interactions, showing for the first time that infection increases host attractiveness to vectors.

Supported by: CAPES
Keywords: Trypanosoma rangeli;Trypanosoma cruzi;Attraction.

HP – 055 - **Development of a lateral flow immunoassay for *Trypanosoma vivax* diagnosis based on a chimeric antigen from African and South American strains**

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Bovine trypanosomosis, caused by *Trypanosoma vivax* (*T. vivax)*, is a major cause of productivity and economic losses in sub-Saharan Africa and Latin America, with increasing reports from new regions. In South America, the absence of systematic diagnostic screening at borders with endemic countries enables the parasite to spread into previously disease-free areas. It is vital to be able to make rapid and accurate diagnoses in the field in order to ensure that treatment can be administered in a timely manner and to reduce the risk of transmission. In this study, we designed a chimeric antigen (QIAFAM) combining conserved epitopes from invariant surface glycoproteins (ISG) of African and South American *T. vivax* strains. QIAFAM was expressed in *E. coli* and subsequently purified to ensure high homogeneity. Its diagnostic performance was assessed via indirect ELISA using 212 bovine sera (149 positive, 63 negative). In comparison to a mixture of individual antigens (TvISGAf + TvISGAm), the QIAFAM-ELISA demonstrated higher sensitivity (95.3% vs. 89.6%) and comparable specificity (90.6% vs. 93.8%), likely attributable to increased epitope availability and reduced steric hindrance in the chimeric design. A lateral flow immunoassay (LFA) in double-paratope format was developed using QIAFAM. In a blinded validation, the LFA achieved 100% sensitivity and specificity on 64 sera (32 positive, 32 negative). In a longitudinal analysis of nine naturally infected and treated cattle, the LFA detected 7/9 cases at day 0, 8/9 at day 15, and 1/9 at day 30 post-treatment, demonstrating comparable performance to ELISA. In contrast to other *T. vivax* LFAs developed with antigens tested only on African samples, our device incorporates a chimeric antigen validated with South American sera, enhancing its geographic applicability. This design supports its potential use for epidemiological surveillance and outbreak response in diverse endemic and emerging regions worldwide.

Keywords: IFL;Diagnosis;Trypanosoma vivax.

HP – 056 - **Unveiling the GP63 Metalloprotease repertoire of Trypanosoma rangeli: From a novel species-specific group to functional insights of secreted and surface-attached glycoproteins**

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Trypanosoma rangeli is a kinetoplastid parasite distributed across the Americas, transmitted by triatomines and infecting diverse mammals. Its phylogenetic proximity to *Trypanosoma cruzi* and its non-pathogenic nature to mammalian species make it a model for comparative studies. We identified 76 *Tr*GP63 genes containing the full-length peptidase M8 domain and classified them into 12 groups, including a novel species-specific group (G12) that is absent in other kinetoplastids. On the other hand, *T. rangeli* lacks the presence of ortholog *Tc*GP63 group 1 (the most conserved group, which includes all *Leishmania* GP63 genes), despite having conserved flanking genes. Most *Tr*GP63 proteins are predicted to be GPI-anchored to the parasite surface; however, members of groups 2, 6, and 8 are likely to be preferentially secreted, as verified by secretome data. Metalloprotease activity was detected in epimastigotes and trypomastigotes, peaking at pH 5.5, but activity levels are lower than those in *T. cruzi* or *Leishmania braziliensis*. Structural modelling of a representative for each *Tr*GP63 group confirmed conserved Zn^{2+}-binding motifs in most sequences, even though only a few structures exhibited the S1' hole in the surface analysis. The analysis of interaction potential of the active sites indicated that 7 out of 15 *Tr*GP63 exhibited a ligand cavity probability above 50%, with sequences from groups 4 and 6 (71% and 74%, respectively) showing values close to the *Leishmania major* control structure (76%). Docking with 1,10-phenanthroline confirmed strong inhibitor orientation in highly promising groups, highlighting potential for functional exploration. This work provides the most comprehensive GP63 repertoire analysis for *T. rangeli*, uncovering species-specific diversity. Although *T. rangeli* lacks representatives of group 1, other proteins, mainly those from groups 4 and 6, may perform equivalent biological functions and represent potential candidates for functional substitution.

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Keywords: Metalloprotease;GP63;Trypanosoma rangeli.

HP – 057 - **Investigation of the effect of lipopeptides from *Bacillus subtilis* on Tegumentary Leishmaniasis: a promising therapeutic approach**

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Leishmaniasis are neglected diseases caused by protozoa of the genus *Leishmania*, affecting over one million people annually, with the tegumentary form accounting for more than 90% of cases. Due to the limitations and adverse effects of available treatments, there is an urgent need for new therapies. In this context, lipopeptides—peptides conjugated to a lipophilic moiety—stand out as a promising alternative, exhibiting antiviral, antifungal, and antimicrobial activities. Produced by various bacterial genera, such as *Bacillus*, they are categorized into three main families: fengycins, iturins, and surfactins. Considering the demand for new therapeutic agents against leishmaniasis, this study evaluated the leishmanicidal potential of a pool of lipopeptides derived from a *Bacillus subtilis* isolate against a causative species of the tegumentary form. The pool, containing iturin, fengycin, and surfactin, was first assessed for cytotoxicity in RAW 264.7 macrophages treated for 48 hours with concentrations ranging from 300 µg/mL to 0.073 µg/mL, using the resazurin assay to evaluate cell viability. Subsequently, macrophages were infected with *Leishmania amazonensis* PH8 parasites at a 20:1 ratio and treated for 48 hours. Parasite viability was then assessed by the resazurin assay, based on the recovery of promastigotes after macrophage lysis. All tested concentrations, except 300 µg/mL, maintained macrophage viability above 75%. In the infection assay, there was a reduction greater than 50% in parasite viability at concentrations of 150, 75, and 37.5 µg/mL, with the greatest reduction observed at 150 µg/mL (4.72%). Further studies are necessary to evaluate its efficacy and selectivity, potentially representing a new strategy for the treatment of tegumentary leishmaniasis.

Supported by: FAPEMIG, CNPQ, CAPES
Keywords: Leishmaniasis;Treatment;Lipopeptides.

HP – 058 - **Exploring molecular mechanisms of vertical transmission in Chagas disease**

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Chagas disease is a parasitic infection caused by the protozoan *Trypanosoma cruzi*. In nature, the parasite is primarily transmitted through two main routes: triatomine vector-dependent transmission, either through contamination with feces from infected triatomine bugs or oral ingestion of contaminated food, and vector-independent transmission, mainly via the transplacental (vertical) route. The transplacental route has gained epidemiological relevance, especially in non-endemic regions and areas where vector control programs have been successful. Interest in studying vertical transmission has increased due to the rising number of congenital Chagas disease cases reported in non-endemic countries, especially in Europe, as a result of migration from endemic regions. Natural *T. cruzi* strains that have been vertically transmitted across three generations were isolated and characterized in Uruguay. We have completed the first phase of characterization of these naturally adapted, vertically transmitted strains (TcVT), both ***in vitro*** and ***in vivo,*** with a primary focus on the host response of mice infected with to TcVT and reference strains. Our data indicate that TcVT strains are highly specialized for congenital transmission and exhibit distinct biological and immunological profiles compared to classical reference strain. In this study, we propose to further characterize these isolates at both the **genomic** and **transcriptomic** levels to better understand the mechanisms underpinning this silent mode of transmission. Using PacBio HiFi sequencing, we have generated high-quality genome assemblies of the TcVT strains, as well as vector-transmitted strains (BolFc10A and TcAR-SE23C). Additionally, transcriptomic analyses are currently underway to identify differentially expressed genes between these strains, focusing on features associated with immune modulation and placental invasion. This integrative approach will help elucidate how these strains modulate the host immune response to establish persistent infection while enabling efficient transplacental passage.

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Keywords: Chagas disease;transcriptomics;vertical transmission.

HP – 059 - **Virulent RH strain of *Toxoplasma gondii* inhibits nitric oxide production through different iNOS pathways in mouse peritoneal macrophages from distinct microenvironmental condition**

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Toxoplasma gondii is an obligate intracellular parasite that infects nucleated cells, including 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* may 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 two different types of macrophages: 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 of *T. gondii*. Both types of macrophages, obtained by peritoneal lavage, were seeded and activated with interferon-γ and lipopolysaccharide. After 24h, macrophage was 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. After 24h of infection with the RH strain, NO production and iNOS+ macrophages reduced in ResMO. In infected StMO, NO production reduced, but iNOS+ macrophages did not alter. *T. gondii* growth was higher in ResMO than in StMO. Infection with the ME-49 strain did not reduce NO production and the parasite was killed independently of the macrophage type. In ResMO, iNOS+ population increased. In StMO, iNOS+ population did not change. Therefore, only the virulent RH strain inhibited NO production degrading iNOS in ResMO, as in some macrophage lineages examined so far, but in StMO, virulent RH strain inhibits NO production without degrading iNOS.

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Keywords: Toxoplasma gondii;peritoneal macrophages;iNOS.

HP – 060 - **IN VIVO ANTI-Plasmodium chabaudi EFFECT OF FeBS METALLOCOMPLEX**

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Malaria, caused by protozoa of the genus *Plasmodium*, has high global mortality, with a higher incidence in tropical and subtropical regions. Resistance to current drugs, such as artemisinin derivatives and quinine, reinforces the need for new therapeutic compounds. This study evaluated the effect of the metallocomplex FeBS in the treatment of experimental *Plasmodium chabaudi* malaria (strain AJ) in *Balb/c* mice. The compound was administered intraperitoneally (500 and 750 µg/kg), and clinical manifestations, survival, parasitemia, and liver and spleen histology were monitored. Treated groups presented later clinical manifestations compared to the control group. In the Kaplan-Meier survival curve, all animals of the control group died by day 12 post-infection; animals of the chloroquine group and FeBS groups died by day 15, with no difference between the FeBS dosages used. The treated groups showed a delay in the progression of parasitemia when compared to the control group; the group treated with chloroquine showed greater parasitemia in relation to both treatments with FeBS . In splenic histology, the negative control showed marked loss of organization; chloroquine and FeBS partially preserved the structure, with the 750 µg/kg dose being the most protective. In the liver, the control showed intense disorganization and abundant hemozoin; chloroquine partially reduced the damage; FeBS, especially at 750 µg/kg, maintained greater tissue integrity and reduced hemozoin deposition. FeBS presented a partial antimalarial effect, delaying clinical manifestations, the progression of the infection, preserving splenic and hepatic structures and prolonging survival, suggesting therapeutic potential to be explored in future studies.
Supported by: CAPES 001
Keywords: Chemotherapy;Metallocomplex;Plasmodium.

HP – 061 - **Target-Based Screening Reveals Pyocyanin as a Potential Anti-*Toxoplasma* Compound**

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Introduction: Toxoplasmosis, caused by the protozoan *Toxoplasma gondii*, affects approximately one-third of the global population. Although many infections are asymptomatic, the disease can cause severe complications in neonates and immunocompromised individuals. Current treatments have limited efficacy and significant toxicity, making the search for new therapeutic options imperative. The identification of therapeutic targets—such as proteins, metabolic pathways, or parasite-essential structures—is considered a promising strategy for developing specific inhibitors. **Objective:** To identify therapeutic targets and evaluate novel inhibitors with potential activity against *T. gondii*. **Methods:** The identification of *T. gondii*-specific enzymes and proteins was performed using the KEGG platform. Potential inhibitors were selected using the BRENDA platform, followed by assessment of ADMET properties and verification of novelty through SwissADME, OSIRIS, and PubMed. The anti-*T. gondii* activity was evaluated against intracellular tachyzoites (RH-2F1 strain) after 72 hours of incubation. **Results:** *In silico* analyses identified 13 potential targets and 40 inhibitors, of which 25 showed predicted inhibitory effects on *T. gondii*-specific enzymes from folate and terpenoid pathways, and 14 on DNA topoisomerase II-alpha and tubulins. Aminodeoxychorismate synthase from the folate pathway, and its inhibitor pyocyanin, stood out for their predicted favorable gastrointestinal absorption, blood–brain barrier penetration, and absence of toxicity. Pyocyanin showed an EC_{50} of 5.84 ± 1.54 µM, while pyrimethamine presented an EC_{50} value of 0.325 ± 0.04 µM. **Conclusion:** The *in silico* screening strategy allowed the identification of pyocyanin as a potential *T. gondii* inhibitor, with a favorable ADMET profile. Preliminary *in vitro* results indicate micromolar anti-*T. gondii* activity. Further cytotoxicity evaluation will be essential to confirm the compound's therapeutic potential.
Supported by: FAPESP (process number 2018/18954-4)
Keywords: Toxoplasmosis;drug discovery;metabolic pathways.

HP – 062 - **The ME-49 strain of *Toxoplasma gondii* is destroyed in the HD11 chicken macrophage lineage, whereas the RH strain persists**

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Toxoplasma gondii is an intracellular pathogen that causes toxoplasmosis and is capable of infecting warm-blooded vertebrate hosts. Strain virulence varies among hosts, and more virulent strains can evade cellular microbicidal systems, surviving and causing disease. Cellular microbicidal systems also differ between hosts, which helps explain the existence of distinct strains. To better understand strain diversity and varying virulence, we investigated the interaction of the parasite with chicken macrophages of the HD11 cell line activated with IFN-γ. HD11 cells were infected with *T. gondii* strains (RH or ME-49) for 2 h and 24 h. Nitric oxide production by HD11 was assessed. Immunofluorescence of infected cells was used to determine parasite persistence. Nitric oxide production was inhibited by infection with the RH strain and remained unchanged with ME-49. After 24 h of infection, the RH strain remained intact but did not divide, indicating that it persisted in activated HD11 cells. In contrast, ME-49 was destroyed after 24 h of infection. The persistence of the RH strain may be related to its ability to inhibit nitric oxide production. These results establish infection parameters for HD11 macrophages, enabling *in vitro* investigation of chicken macrophage microbicidal mechanisms and helping to understand how different parasite strains bypass these defenses, potentially clarifying susceptibility and resistance among different strains in chickens.
Keywords: Toxoplasma gondii;macrophages;chickens.

HP – 063 - **Effects of glycolysis inhibition on growth and mitochondrial respiration of *Trypanosoma cruzi* epimastigotes cultured with heme**

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Trypanosoma cruzi, the etiological agent of Chagas disease, is a protozoan transmitted through the feces of the triatomine vector. The epimastigote forms of *T. cruzi* proliferate into the midgut of insect, in contact with molecules derived from the digestion of blood, such as heme. Heme is a metalloporphyrin that, when dissociated from hemoglobin, becomes a potent oxidizing agent. A transcriptomic analysis performed by our group revealed that several enzymes involved in *T. cruzi* energy metabolism were upregulated in the presence of heme, highlighting the significant role of this molecule in the physiology of this stage of the parasite. One of these enzymes was hexokinase, which plays a key role in the glycolytic pathway. Therefore, the aim of this study is to investigate the importance of heme and glucose metabolism for the proliferation and energy metabolism of *T. cruzi* epimastigotes, using a glycolysis inhibitor, 2-deoxy-D-glucose (2-DG). The results showed that the proliferation of *T. cruzi* epimastigotes was highly increase in the presence of heme. However, glycolysis inhibition by 2-DG reduced this heme-stimulated proliferation by 74%. Despite this effect on growth, ATP content was similar between both conditions, while parasites treated with 2-DG alone showed a 28% increase in ATP levels. Interestingly, parasites with inhibited glycolysis seem to increase their hexokinase activity after 3 days of incubation with heme. Regarding mitochondrial respiration, treatments with 2-DG addition increase both routine and maximal respiration, as well as raise the Oxygen consumption through oxidative phosphorylation. These data indicate that, when glycolysis is inhibited, mitochondrial respiration is stimulated to sustain ATP production in *T. cruzi* epimastigotes. However, this compensation does not result in increased proliferation, indicating a high dependence on glucose.
Supported by: FAPERJ, CAPES, CNPq and INCT-EM
Keywords: Trypanosoma cruzi;Glycolysis;Heme.

HP – 064 - **The Role of NINJ1 in Immune Modulation and Lytic Cell Death During *Toxoplasma gondii* and *Neospora caninum* Infections**

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Ninjurin-1 (NINJ1) is a plasma membrane protein with two transmembrane domains that mediates complete membrane rupture (PMR) during apoptosis, pyroptosis, or necrosis, enabling the release of large molecules such as lactate dehydrogenase (LDH). Its role in protozoan parasitic infections, however, remains poorly defined. This study aimed to determine whether *Toxoplasma gondii* and *Neospora caninum* induce lytic cell death in bone marrow–derived macrophages (BMDMs) and to elucidate the roles of caspase-1 and NINJ1 in plasma membrane permeabilization and rupture during infection. Using wild-type (WT), caspase-1 knockout (caspase-1$^{-/-}$), and NINJ1 knockout (Ninj1$^{-/-}$) BMDMs, we measured PI uptake, LDH release, parasite replication, inflammatory cytokine production, NINJ1 expression, and caspase-1 activation. Both parasites induced significant lytic cell death in WT BMDMs, evidenced by increased PI uptake and LDH release, effects that were markedly reduced in NINJ1-deficient cells. Uninfected cells-maintained baseline membrane integrity. NINJ1 expression increased following infection, including in caspase-1$^{-/-}$ BMDMs, suggesting transcriptional regulation via caspase-1–independent pathways. Differences in NINJ1 expression between parasites were not statistically significant. caspase-1 activation was similar between WT and NINJ1-deficient cells for both infections, indicating that NINJ1 does not modulate Caspase-1 activation. These findings identify NINJ1 as a key effector of plasma membrane rupture in apicomplexan infections and highlight its potential as a therapeutic target for modulating host–pathogen interactions.

Supported by: CAPES, FAPEMIG, CNPq
Keywords: Ninjurin-1;Lytic cell death;Apicomplexan parasites.

HP – 065 - ***Leishmania amazonensis* infection reprograms human macrophage lipid metabolism by inducing triacylglycerol accumulation and fatty acid profile remodeling**

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Parasites of the genus *Leishmania* manipulate host cell metabolism for survival and replication. Lipid droplets (LDs), key organelles in lipid homeostasis, are crucial, but the host's global lipid profile modulation remains unclear. We characterized lipid metabolism alterations in human macrophages (THP-1) after *Leishmania amazonensis* infection for 48h. Thin-layer chromatography revealed a massive accumulation of storage lipids—mainly cholesteryl esters, diacylglycerols and triacylglycerols (TAG)—at 24 and 48 hours, a result visually confirmed by increased LDs via confocal microscopy with BODIPY. To understand this accumulation's origin, uptake and synthesis pathways were investigated. Surprisingly, the ability of macrophages to uptake and maintain cholesterol-^{3}H from external lipoproteins (LDL and HDL) was diminished at 48h post-infection. This indicates the massive TAG and cholesteryl ester accumulation is not from an increased external palmitate flux, suggesting use of other sources for *de novo* lipogenesis. GC/MS analysis of the fatty acid (FA) composition expanded these findings, revealing an increase in palmitic (C16:0), stearic (C18:0), and oleic acid (C18:1) in infected cells. Infection also expressively remodeled the omega-6 fatty acid profile, with emergence of key inflammatory precursors: Arachidonic Acid (C20:4), Dihomo-γ-Linolenic Acid (DGLA) (C20:3), and Osbond Acid (C22:5). The availability of lipids in the culture medium greatly influences lipid accumulation in macrophages. This lipid biosynthesis increase during *Leishmania* infection is an impressive metabolic shift, as the parasite induces the host cell to synthesize lipids far beyond its basal capacity. In conclusion, *Leishmania amazonensis* drives a complex reprogramming of macrophage lipid metabolism beyond energy storage, actively modulating the FA profile, likely creating a favorable intracellular environment and possibly modulating the host's immune response.

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Keywords: Lipid metabolism;Leishmania amazonensis;Fatty acid reprogramming.

HP – 066 - ***Trypanosoma cruzi induces* host nuclear remodeling and *DNA damage response* through infection and extracellular vesicles**

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Trypanosoma cruzi, the causative agent of Chagas disease, affects 8 million of people worldwide. Despite extensive research, the impact of *T. cruzi* on host nuclear dynamics remains poorly understood. We demonstrated that *T. cruzi*infection modulates the host cell nucleus by downregulating RNA polymerase II activity and associated RNA-binding proteins, including hnRNPA1, hnRNPA2B1, and the splicing factor U2AF35, leading to impaired host transcription and RNA processing. Additionally, infection induces host DNA damage, evidenced by double-strand breaks (DSBs) and activation of the DNA damage response (DDR), marked by phosphorylation of H2AX, 53BP1, and ATM in non-phagocytic epithelial LLC-MK2 cells. We observed a temporal activation of the DDR pathway, with peak of DNA damage at 12 hours post-infection, followed by repair mechanisms primarily through the non-homologous end joining (NHEJ) pathway mediated by DNA-PK. To assess whether parasite internalization is required for this effect, we exposed LLC-MK2 cells to extracellular vesicles (EVs) derived from *T. cruzi* trypomastigotes. EVs were internalized in a time- and dose-dependent manner triggered DNA fragmentation and activation of DDR proteins, including γH2AX,γATM, and γDNA-PK, without affecting cell cycle progression. These findings reveal that *T. cruzi* can alter host nuclear function and genome integrity both directly and via secreted EVs, highlighting novel mechanisms of host-pathogen interaction and potential targets for the therapeutic intervention.
Supported by: FAPESP, CAPES, FAEPA
Keywords: Trypanosoma cruzi;DNA damage response ;extracellular vesicles .

HP – 067 - **EXPERIMENTAL TECHNIQUES TO DEVELOP NEXT GENERATION DRUG LIKE MOLECULES FOR NEGLECTED DISEASES - LEISHMANIASIS**

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World Health Organization defines Neglected Tropical Diseases as a heterogeneous group of diseases prevalent in tropical and subtropical areas of 149 countries and affecting more than 1 billion people, bringing a high social and economic cost to developing countries. Tropical diseases such as malaria, Chagas' disease, sleep sickness and Leishmaniasis remain as some of the leading causes of morbidity and mortality worldwide. Leishmaniasis is a worldwide anthropozoonosis, being endemic in 98 countries distributed in Africa, Asia, Europe and America. It is estimated that 12 million people have already been infected with the disease and between 0.9 and 1.6 million new cases occur each year. The strategies for leishmaniasis preventive control have been ineffective in view of the increase in the number of cases and the appearance of cases in urban centers in Brazil and in several regions of southern Europe. The drugs that are on the market present a series of problems, such as resistance of the parasite and induction of adverse side effects, that limit their use and mainly its effectiveness. In addition, all drugs available in Brazil and most of the countries affected are of parenteral administration, which leads to the low adhesion to the treatment and favors the emergence of resistant strains. Our goal is to synthesize innovative compounds with high leishmanial potential. The compound NGB30.0002 is a chlorovinylaldehyde derivative, its infrared and mass spectrum were recorded, and tested against promastigotes of *L. amazonenssis, L brasiliensis* and *L. major* in the presence of different concentrations of the molecules (100, 50, 25, 12.0 and 6 µg/ml). Viable cells were counted after 72 h of incubation and the percentage inhibition of *L. amazonensis* after incubation with the compound was 86.9, 67.2, 48.4, 31.3 and -12.5, for *L. brasiliensis* was 98.1, 97.2, 95.3, 62.5 and 15.6 and for L. major was 96.4, 82.2, 43.3, 7.8 and -10, respectively, showing considerable activity.
Supported by: PIPE-FAPESP 2020/01465-0
Keywords: Leishmanicidal activity; Neglected Disease;Drug Like.

HP – 068 - **EVALUATION OF THE BLOOD-RETINAL BARRIER COMPONENTS IN A MURINE MODEL OF CONGENITAL TOXOPLASMOSIS**

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Toxoplasmosis is caused by infection with *Toxoplasma gondii*. Congenital *T. gondii* infection is the most severe form of toxoplasmosis and can cause serious harm to the fetus, such as spontaneous abortions, intracranial calcification and hydrocephalus. CT can also lead to visual defects, which involve infection and damage to structures of the visual system, such as the retina. During retinal development, angiogenesis is essential for the formation of a structure that is a specialized extension of the blood-brain barrier, named Blood-Retina Barrier (BRB). The BRB is composed of cell-cell junctions at endothelial cells and tightly regulates the exchange of nutrients and metabolites between the blood and the neural tissue. Our aim is to evaluate BRB components in a congenital toxoplasmosis model. Pregnant C57BL/6 mice received an intragastric inoculation of two *T. gondii* cysts on embryonic day 10 (E10); the control group received an injection of brain macerate from an uninfected mouse. Animals were euthanized on postnatal day 5 (P5), and the retinas and brain were dissected for immunohistochemistry, RT-qPCR, and Western blotting analyses. We detected bradyzoite RNA in the retinas and brains of mice from infected dams at P5, confirming the vertical transmission. Higher bradyzoite signal was found in the brains than in the retinas. Infected mice retinas had a significant decrease in content of glial fibrillary acidic protein (GFAP), an astrocyte marker, in addition to a decrease on its immunoreactivity observed by confocal microscopy. Levels of tight junction proteins claudin 5 were decreased, whereas occludin had a trend to increase. Our data points to a possible influence of congenital *T. gondii* infection on retinal components, which are important for the angiogenesis and blood-retinal barrier development, including astrocytes and tight junctions' integrity.
Supported by: FAPERJ, Capes, CNPQ, INOVA Fiocruz
Keywords: Toxoplasma gondii;Blood-retinal barrier;Congenital toxoplasmosis.

HP – 069 - ***Leishmania amazonensis* promatigotes induce human macrophages to release DNA extracellular traps (DETs)**

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Leishmaniasis is a neglected disease caused by protozoa from the genus *Leishmania*, affecting around 12 million people worldwide, and presents different clinical manifestations. *Leishmania amazonensis* is the agent of cutaneous leishmaniasis in the New World, with a proportion of cases evolving to the severe diffuse cutaneous leishmaniasis. Promastigotes inoculated by a sand fly vector into the skin interact with the innate immune cells of the vertebrate host and establish themselves in macrophages. It has been shown that macrophages release DNA extracellular traps (DETs) in response to various stimuli. These structures are composed of DNA, histones, and antimicrobial proteins, and are capable of immobilizing and neutralizing pathogens. This study aims to investigate the ability of human macrophages to release DETs in response to the interaction with *L. amazonensis*, as well as to characterize their composition, morphology, functionality, and the signalling pathways involved. To this end, primary macrophages were differentiated from PBMCs over 7–10 days in RPMI medium with 5% human serum. Promastigotes from the MHOM/BR/75/Josefa strain were maintained in Schneider's medium supplemented with 10% fetal bovine serum at 26°C. In the assays, macrophages were incubated with *L. amazonensis* at different ratios and for different times. Supernatants, obtained after centrifugation, had their DETs quantified using PicoGreen. Our results show that human macrophages release DETs in response to *L. amazonensis* at the different ratios tested. DET release was confirmed by DNase treatment. We further demonstrate that DET induction by *L. amazonensis* depends on phagocytosis and TLR-2, but occurs independently of reactive oxygen species production.
Supported by: CNPQ, CAPES e FAPERJ
Keywords: Leishmaniasis;Macrophages;DETs.

HP – 070 - **Toxoplasma gondii infection leads to epigenetic reprogramming in mouse neuroblast cells and the developing brain**

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Vertical transmission of *Toxoplasma gondii* during pregnancy can lead to severe malformations, and our group has shown that infection of neural progenitor cells impairs neurogenesis. The neurogenic program is regulated by epigenetic marks that influence chromatin accessibility and cell fate. *T. gondii* secretes effector proteins, such as TEEGR (Toxoplasma E2F4-associated EZH2-inducing gene regulator), that modulate histone 3 (H3) methylation and may contribute to parasite persistence. We investigated how *T. gondii* infection modulates epigenetic marks associated with active (H3K4me3) and repressed (H3K27me3) chromatin states *in vitro* and *in vivo*. Neuro2a cells were infected with RH (type I), ME49 (type II), or VEG (type III) strains, and H3K4me3 and H3K27me3 levels were evaluated by western blot at 12, 24, and 96 hours post-infection (hpi). Immunofluorescence was performed to assess nuclear signal intensity. *In vivo*, pregnant Swiss Webster mice were infected intragastrically with ME49 at the 10th day of pregnancy, and embryonic brain cortices were collected at E15 and E17 for western blot and qPCR analysis of methyltransferase genes (Rbbp5, Ash2l, Setd1a, Setd1b, Ezh1, Ezh2). In Neuro2a cells, H3K27me3 nuclear fluorescence increased upon infection with all strains, while H3K27me3 protein content increased significantly in ME49-infected cells at 24 hpi. H3K4me3 levels increased in RH-infected cells at 12 hpi. In embryonic brain cortices, ME49 infection led to reduced brain size, Ezh1 and Ezh2 expression at E17 compared to controls, with Setd1b showing a trend to increase at E15 and a significant increase at E17. Other methyltransferase genes showed no significant change. These findings suggest that *T. gondii* induces strain-specific and stage-specific epigenetic reprogramming in neural cells, both in vitro and during brain development, which may contribute to the neurodevelopmental alterations associated with congenital toxoplasmosis.

Supported by: FAPERJ E FIOCRUZ
Keywords: Toxoplasmosis;neurodevelopment;epigenetic reprogramming.

HP – 071 - ***In silico* screening of possible drugs targeting *Leishmania braziliensis* SRPK using molecular dynamics and molecular docking**

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Current treatments for cutaneous leishmaniasis are prolonged, often needing frequent visits to a professional for administering. As they are highly toxic, the treatment require constant monitoring of liver, heart and kidney function, which in many cases results in treatment abandonment and failure. The development of new drugs which are more effective, less toxic and cheap poses as a priority in the fight against leishmaniasis and neglected diseases in general. However, the high cost of development of a new candidate from scratch that meets these criteria disincentivize the pharmaceutical industry due to low profit margins. On the other hand, by applying drug repurposing the total cost of taking a drug from lab to shelf can be cut to a tenth. One of the main techniques for drug repurposing is the use of *in silico* screening of large databases to determine the best candidates against a specific target, which is less expensive and less labor intensive than testing all then *in vitro*. In this work, the *Leishmania braziliensis* SRPK protein structure was modeled using CollabFold and molecular dynamic was performed using OpenMM in a cloud-based notebook. The resulting trajectories file was clustered using CLoNe in two major poses (Cluster 1 and Cluster 2) that best represent the protein structure. The clusters were then docked on GOLD against the PKIDb database of kinase inhibitors and ranked using ChemPLP scoring. The two best ranked compounds for Cluster 1 (1A and 1B) and for Cluster 2 (2A and 2B) were then analyzed in complex with the protein to map protein-ligand interactions. Although virtual screening is not enough to determine if a molecule will have effect as a treatment for a disease, it can be used to filter out and focus research efforts, increasing the chances of success in discovery or repositioning of new drugs. Of these compounds, 2A is currently used for cancer treatment in dogs on the European Union and is commertialy available for testing *in vitro*.

Supported by: FAPEMIG, CAPES, CNPq
Keywords: Leishmaniasis;Virtual Screening;Drug Repurposing.

HP – 072 - **Role of the transcriptional repressor factor Enhancer of Zeste Homolog 2 (EZH2) in *Leishmania (Leishmania) amazonensis* infection**

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Intracellular parasites, including Leishmania, are equipped with an arsenal of molecules that modulate the epigenetic state and gene expression of their hosts, and the availability of these molecules to the host can occur through the secretion of EVs. The enzyme Enhancer of Zeste 2 (EZH2), responsible for the repressive epigenetic marker H3K27me3, regulates essential biological processes. However, its role during L. amazonensis infection remains unknown. This study aims to test the hypothesis that L. amazonensis infection induces repressive epigenetic modifications in macrophages, evaluating the role of the EZH2 enzyme and the contribution of Extracellular Vesicles (EVs) in modifying the macrophage epigenetic landscape. EVs were purified from L. amazonensis promastigotes at 35°C, and scanning and transmission electron microscopy demonstrated budding of these EVs and their typical structure. The predominant size was approximately 100 nm. Using Western blotting, an increase in H3K27me3 was observed with L. amazonensis infection and also with EV treatment for 5 hours. An 18-hour period showed a decrease in this marker. Notably, EZH2 expression increased during infection. Inhibition of EZH2 by GSK-343 in THP1 macrophages led to a reduction in parasite load. Inhibition of EZH2 with GSK-343 or Tazemetostat led to demethylation of H3K27me3 even after infection. HDAC1 and DNMT1 levels were assessed by real-time PCR, which demonstrated increased levels of their respective mRNAs during infection or EV exposure. EZH2 can recruit DNA methyltransferases and histone deacetylases to silence genes that subvert the leishmanicidal response of macrophages. These data suggest that EZH2 plays an essential role in the outcome of Leishmania infection and that its inhibition may be a promising therapeutic strategy to reduce parasite load and control infection.

Supported by: FAPERJ, CNPq
Keywords: Epigenetic;trimethylation;Leishmaniasis.

HP – 073 - **ARGINASE 1 INDUCES THE GROWTH OF *Toxoplasma gondii* IN RAW 264.7 MACROPHAGES ACTIVATED IN THE M2 PROFILEARGINASE 1 INDUCES THE GROWTH OF *Toxoplasma gondii* IN RAW 264.7 MACROPHAGES ACTIVATED IN THE M2 PROFILE**

PAULA, L.D.S.; CABRAL, G.R.D.A.; XAVIER JUNIOR, J.T.; DE SÁ, A.B.M.; ARAÚJO, S.E.S.; SEABRA, S.H.; DAMATTA, R.A..
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Macrophages (MØs) are immune cells with distinct activation phenotypes: M0 (resident), M1 (microbicidal, inflammatory), characterized by the production of nitric oxide by the enzyme inducible nitric oxide synthase, and M2 (tissue homeostasis, anti-inflammatory), highly expressing arginase 1 (ARG1). ARG1 produces essential molecules required for polyamine synthesis, important for cell proliferation and potentially contribute to parasite's survival. This study evaluated the role of ARG1 in RAW 264.7 MØs to *T. gondii* development. RAW 264.7 MØs on M0 and M2 profiles were infected with *T. gondii* (RH strain) and ARG1 activity, expression, localization and parasite development were evaluated. ARG1 activity was analyzed after activation with different concentrations of 8-Bromoadenosine 3′:5′-cyclic monophosphate (BrAMPc) and Interleukin-4 (IL-4). The optimal maximum enzymatic ARG1 activity in M2 MØs was achieved. These M2 MØs presented higher ARG1 expression compared to M0 MØs. Immunofluorescence reveled that ARG1 was localized in vesicles concentrated at the plasma membrane of M2 MØs. In M0 MØs ARG1 was not detected. However, when M2 MØs were infected with *T. gondii*, ARG1 migrated to the parasite's parasitophorous vacuole. Infection of *T. gondii* after 2 h was similar between M0 and M2 MØs. After 24 and 48 h, M2 MØs treated with ARG1 inhibitor presented fewer parasites than untreated M2 MØs. M0 MØs also presented fewer parasites following ARG1 inhibitor treatment, but to a lesser extent than M2 MØs. The localization of ARG1 to the parasite parasitophorous vacuole, along with the reduced development of *T. gondii* following ARG1 inhibition, indicates that ARG1 positively contributes to parasite growth and maintenance. These findings suggest that ARG1 play an essential role in the development of *T. gondii* within macrophages.

Keywords: Toxoplasma gondii;Arginase 1;Macrophages.

HP – 074 - **Xylose synthesis deficiency in Trypanosoma cruzi reveals an unexpected interplay between activity and glycosylation for the flagellar attachment zone protein GP72**

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Glycoconjugates are well-established virulence factors in trypanosomatids, which are responsible for major neglected human diseases. Of the three trypanosomatids of medical importance, *Trypanosoma cruzi* - the etiological agent of Chagas disease - is the only one to contain xylose residues, which are found on TcGP72, a highly glycosylated protein associated with flagellar adhesion. Xylose is incorporated into glycoproteins from UDP-xylose (UDP-Xyl), which is synthesized from UDP-glucose in two steps. UDP-glucuronic acid (UDP-GlcA), produced by the action of UDP-glucose dehydrogenase (UGD), serves as an intermediate in this pathway. To investigate the role of xylose metabolism in *T. cruzi*, we generated a *Tcugd* null mutant unable to synthesize UDP-GlcA and, consequently, UDP-xylose. Knockout of *TcUGD* rendered *T. cruzi* cells unable to synthetize UDP-GlcA and UDP-Xyl as demonstrated by mass spectrometry analyses. Proliferation of *Tcugd* epimastigotes in LIT medium was not affected; however, reduced differentiation rates into infective metacyclics were observed. A monoclonal antibody (WIC29.26), which recognizes a glycan epitope on TcGP72 and other glycoproteins, failed to react with total protein extracts obtained from the mutant cells. This result is not surprising, given the presence of xylose on the complex glycan moiety of TcGP72. However, flagellar detachment from the cell body - which is characteristically observed in *Tcgp72* knockout mutants - was not detected in the *Tcugd* mutant. Endocytosis of transferrin and BSA, however, which is not altered in *Tcgp72* null mutants, was found to be reduced in *Tcugd* -/- mutants. These results suggest that proper glycosylation of TcGP72 may not be essential for its role in flagellar adhesion, highlighting an apparent complex relationship between TcGP72 activity and its glycosylation pattern.
Supported by: FIOCRUZ
Keywords: Trypanosomatids;Glycosylation;Flagellar adhesion.

HP – 075 - **Effect of Pirfenidone on fibrosis induced by *Trypanosoma cruzi* infection in cardiac spheroids/organoids**

SEYDEL, C.M.; COELHO, L.L.; DA SILVA, D.M.; GARZONI, L.R.
. FIOCRUZ/IOC, RIO DE JANEIRO - RJ - BRAZIL.

Chagas disease (CD) is a systemic illness caused by *Trypanosoma cruzi* (*T. cruzi*) with significant morbidity and mortality. Cardiac developments are the most important manifestation of CD, leading to accumulation of fibrotic and hypertrophic tissue in the heart. As so, it contributes to functional abnormalities, heart failure and sudden death, the main cause of mortality among patients with CD. Regardless of the frequency and severity of the cardiomyopathy, there are no effective therapies for the treatment of cardiac fibrosis. Repositioning and combination of known compounds have been strategies openly used in preclinical research. In this way, Pirfenidone is a drug currently used for the treatment of idiopathic pulmonary fibrosis and has demonstrated antifibrotic effects in the cardiac context. In the pre-clinical scenery, culture systems that mimic features of the *in vivo* microenvironments, as tissue architecture and functionality, are interesting models to study novel therapies. Our group established a 3D model that reproduces cardiac fibrosis and hypertrophy when infected with *T. cruzi*. Thus, this project aimed to evaluate the anti-fibrotic effect of Pirfenidone in a 3D model (spheroids/organoids) of cardiac fibrosis induced by *T. cruzi* infection. For that, we investigated the effect of the drug on cell viability, on the infection itself and, mainly, on the extracellular matrix protein fibronectin, related to cardiac fibrosis. Results demonstrated that after treatment, infected spheroids showed a reducing trend on its diameter, which could be associated with the decrease of the deposition of fibronectin, but not its expression. Also, there was no trypanosomicidal effect on infected spheroids/organoids after treatment. In this way, Pirfenidone appears to be a promising therapeutic strategy to continue to be explored for the treatment of cardiac fibrosis in CD.
Supported by: IOC/Fiocruz
Keywords: cardiac spheroids;fibrosis;T;cruzi.

HP – 076 - **Investigation of the Hippo-YAP/TAZ signaling pathway in fibrosis during *Trypanosoma cruzi* infection of cardiac organoids**

COELHO, L.L.; SEYDEL, C.M.; DA SILVA, D.M.; VIANNA, M.M.; GARZONI, L.R..
INSTITUTO OSWALDO CRUZ - FIOCRUZ, RIO DE JANEIRO - RJ - BRAZIL.

Chronic Chagas cardiomyopathy (CCC) is the main clinical manifestation of Chagas disease (CD) and still lacks effective therapies to reduce complications such as progressive fibrosis. Exploring new approaches targeting the pathophysiological mechanisms of CCC is essential. Three-dimensional (3D) models, such as organoids, can more accurately reproduce the architecture and function of tissues *in vivo*. In 2008, our group established an *in vitro* model of cardiac fibrosis and hypertrophy induced by *Trypanosoma cruzi* infection using cardiac organoids, which has been applied to the evaluation of antiparasitic and antifibrotic therapies. The Hippo signaling pathway, through its co-activators YAP and TAZ, is associated with the pathogenesis of cardiovascular diseases, promoting angiogenesis, hypertrophy, and fibrosis; however, its role in CD remains unknown. We aim to investigate the involvement of the Hippo-YAP/TAZ pathway in *T. cruzi*-induced fibrosis in cardiac organoids to identify new therapeutic targets. We have characterized primary cardiac organoids, standardized antibodies for YAP/TAZ and p-YAP in 4T1 tumor cells (positive control) using immunofluorescence and Western blot, and assessed the effect of verteporfin on organoid viability. Organoids exhibited spontaneous contractility and, after 144 h of infection, showed an increase in diameter and extracellular matrix protein expression, reproducing the *T. cruzi*-induced fibrosis model. Verteporfin induced cytotoxicity at high concentrations. Next steps include evaluating YAP/TAZ and p-YAP expression during infection and analyzing the impact of pathway modulation by verteporfin on fibrosis and parasite load, as well as studying the pathway in primary cardiac fibroblasts.
Supported by: Fiotec, Fiocruz
Keywords: Chagas disease;cardiac fibrosis;three-dimensional cell culture.

HP – 077 - **The Impact of the Icoaraci Virus (ICOV) Nonstructural Protein S (NSs) on the Infection by Leishmania amazonensis**

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Leishmania (L.) amazonensis may cause Cutaneous Leishmaniasis (CL), and is the primary species associated with Diffuse Anergic Cutaneous Leishmaniasis in South America. Its transmission to humans occurs primarily in the Amazon region, via Lutzomyia flaviscutellata. Previous studies from our group and others have demonstrated that the antiviral response mediated by the dsRNA-dependent kinase (PKR) during macrophage infection with L. amazonensis induces the expression of Nrf2 and upregulates SOD1, counteracting the effects of ROS. More recently, we showed that coinfection with L. amazonensis and the Amazonian Phlebovirus Icoaraci (ICOV) enhances parasite burden both in vitro and in vivo, while modulating PKR signaling pathways. Phleboviruses are single-stranded RNA viruses composed of three circular segments.
The S segment encodes the nonstructural protein NSs, the major virulence factor of this Phlebovirus, and it is known to suppress host antiviral responses and modulate cellular signaling pathways such as IFN-1 and oxidative stress. For instance, NSs from Rift Valley targets PKR and p62/TFIIH for degradation, while NSs from STSF interacts with TRIM21, activating the p62–KEAP1–NRF2 axis, thereby promoting an antioxidant environment in host cells. We decided to investigate the role of ICOV/NSs in the outcome of Leishmani infection. Our results indicate that NSs alone are capable of increasing IFN-β and IL-10 expression, without affecting IL-12 levels. It also triggers an antioxidant response, evidenced by increased protein and mRNA levels of NRF2, SOD1, and HO-1. NSs-expressing cells display enhanced resistance to hydrogen peroxide and antileishmanial drugs. Importantly, extracellular vesicles derived from these cells contain NSs and further promote L. amazonensis infection in naive macrophages. Respirometry assays revealed elevated oxygen consumption in NSs-expressing cells, along with an increased mitochondrial DNA to nuclear DNA ratio and upregulation of PGC1-α and NRF1 transcripts, indicating activation of mitochondrial biogenesis. In summary, the expression of ICOV/NSs in macrophages led to a strong antioxidative response, induced some markers of mitochondrial biogenesis, and favored Leishmania infection. The investigation of ICOV/NSs proteins in macrophages is in progress.
Supported by: CNPq, FAPERJ
Keywords: Phlebovirus;Leishmania amazonensis;Oxidativo stress.

HP – 078 - **Antileishmanicidal Activity of Dichalcone Derivative NGB 08.0001 against *L. amazonensis***

MELO, T.S.[1]; SAAVEDRA, M.[1]; DE OLIVEIRA, W.V.[1]; DA CONCEIÇÃO JUNIOR, F.[2]; DIAS, T.D.S.[3]; SOARES, D.C.[2]; DA SILVA, L.H.P.[3]; SARAIVA, E.M.[2]; DELORENZI, J.C.M.O.B.[1].
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Leishmaniasis, a clinical disease caused by parasites from the genus Leishmania, affects millions of people in 98 countries. It is estimated that 12 million people have already been infected with the disease and with an estimated 0.9 to 1.6 million new cases each year. Although there is a worldwide health problem, leishmaniasis is considered a highly neglected disease, lacking efficient and low toxic treatment. The strategies for leishmaniasis preventive control have been ineffective in view of the increase in the number of cases and the appearance of cases in large urban centers in Brazil and in several regions of southern Europe. The drugs that are on the market present a series of problems, such as resistance of the parasite and induction of adverse side effects, that limit their use and mainly its effectiveness. In addition, all drugs available in Brazil and most of the countries affected are of parenteral administration, which leads to the low adhesion to the treatment and favors the emergence of resistant strains. Given the severity of disease caused by Leishmania spp. our aim was to investigate the activity of the compound NGB08.0001 cyclohexanecarbonitriles derived from dichalcones, against promastigotes of L. amazonensis and its cytotoxicity. The compound NGB8.0001 showed high activity against the parasites, IC_{80} NGB08.0001 ~ 1µM and high cytotoxicity at 100mM. Although newly synthesized compounds exhibited good results against L. *amazonensis,* further structure modifications should be made to reduce the cytotoxicity. Compound NGB08.0001 exhibited a considerable activity for a hit compound.
Supported by: PIPE-FAPESP 2020/01465-0
Keywords: Antileishmanial activity;Cytotoxicity;Dichalcones derivative.

HP – 079 - **Connecting the dots: RPA and R-loops in Trypanosoma cruzi**

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Trypanosoma cruzi, the etiological agent of Chagas disease, alternates between insect vectors and mammalian hosts, facing diverse microenvironments and immune challenges. This lifestyle demands a balance between generating genetic diversity for adaptation and preserving core genome stability. Although generally accurate, DNA replication can also contribute to variability through replication-transcription conflicts, a process previously linked by our group to mutation hotspots in *T. cruzi*. These conflicts are linked to DNA:RNA hybrids (R-loops), which form when nascent RNA hybridizes with the DNA template strand, displacing the non-template strand. Under physiological conditions, they aid transcription regulation, chromatin organization, and replication initiation, but excess can stall forks and cause breaks. The Replication Protein A (RPA) complex is the main single-stranded DNA-binding protein, acting in replication and repair. In model systems, RPA binds R-loops to prevent secondary structures and recruit resolution factors. While characterizing TcRPA-1 interactors by immunoprecipitation-mass spectrometry, we found enrichment of ribosomal proteins, which may reflect interactions in rRNA-rich contexts such as the nucleolus, a hotspot for R-loops, and ALBA-family proteins, known to modulate DNA:RNA hybrid stability. This led us to hypothesize that RPA could act in R-loop regulation in *T. cruzi*. We then tested this hypothesis by immunofluorescence, which revealed nuclear co-localization of three RPA subunits with R-loops, especially after camptothecin treatment, a topoisomerase I inhibitor that favors R-loop stabilization. Altogether, our results suggest that the RPA complex is recruited to R-loop-rich regions in *T. cruzi*, supporting a possible role for RPA in R-loop-associated processes.
Supported by: FAPESP processo 2021/12872-9
Keywords: T; cruzi;R-loop;RPA.

HP – 080 - ***In vitro* and *in vivo* evaluation of the Zinc Complex associated with Biocurative using 3D printing technology for the treatment of tegumentary ulcers induced by *Leishmania amazonensis***

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Leishmaniasis, classified by the WHO as a neglected tropical disease, affects approximately 1.3 million people annually and puts 310 million at risk. Current therapy presents severe side effects and favors the emergence of resistant strains, making the search for new strategies urgent. Metalocomplexes, compounds with a metal core coordinated to organic ligands, present advantages over conventional drugs, such as different geometries and interactions. Metalocomplexes have already demonstrated activity against *Leishmania spp*. In this study, the *in vitro* effect of the zinc complex on *L. amazonensis* promastigotes treated with (1–80 µM) was evaluated. The IC_{50} showed values between 0.98 and 1.8 µM (24–120 h), inducing ultrastructural alterations compatible with apoptosis, such as DNA condensation and apoptotic bodies, and no relevant toxicity in LLC-MK_2 cells. In the *in vivo* assay, lesions were induced in *Balb/c* mice (CEUA/UENF no. 486) treated with biocurative associated with the zinc complex (100 µg/kg). After 40 days, a significant reduction in lesions was observed and histologically a clear formation of epidermis, dermis, and hypodermis was seen in the treated groups, indicating healing compared to the control and to the group treated with biocurative associated with aphotericin B. These findings reinforce the potential of the zinc complex, associated with the biocurative, as a promising alternative for the development of a new product against tegumentary leishmaniasis, using 3D printing technology.
Supported by: CAPES
Keywords: Leishmania amazonensis;;metalocomplex;;biocurative.

HP – 081 - **Modulation of lipid metabolism in *Rhodnius prolixus* by *Trypanosoma cruzi* infection**

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Rhodnius prolixus is a hematophagous insect and vector of *Trypanosoma cruzi*, the causative agent of Chagas disease. The parasite can synthesize some lipids but also acquires them from its host. To better understand this interaction, we studied changes in lipid metabolism in *R. prolixus* during *T. cruzi* infection. Lipid profile analysis of the hemolymph revealed a significant decrease in esterified cholesterol, hydrocarbons, fatty acids, 1,3- and 1,2-diacylglycerol, and free cholesterol in infected insects. In the fat body, a key organ for lipid metabolism, levels of triacylglycerol and fatty acids were also reduced. Gene expression analysis showed a significant increase in *RpACC*, *RpDGAT-1*, and *RpDGAT-2*, all involved in lipid synthesis. Although the parasite remains restricted to the gut throughout its life cycle in the insect, changes were observed in other tissues, suggesting a still-unknown communication pathway. To explore this, hemolymph from infected insects was injected into uninfected controls. One day later, a reduction in triacylglycerol and fatty acids, along with an increase in monoacylglycerol, was observed in the hemolymph of treated insects. Considering that miRNAs regulate gene expression and can be transported by lipoproteins, we investigated the presence of miRNAs in hemolymph and lipophorin. *rpr-mir-375-3p* and *rpr-mir-281-3p* were detected in both control and infected insects, with higher levels of *rpr-mir-281-3p* in hemolymph and both miRNAs in lipophorin of infected insects. These findings contribute to a better understanding of the interaction between *R. prolixus* and *T. cruzi*.
Supported by: CAPES, CNPq, FAPERJ
Keywords: Rhodnius prolixus;Trypanosoma cruzi;Lipid metabolism.

HP – 082 - **Supplementation with L-arginine on *Trypanosoma cruzi* infection: Effect on cerebral microcirculation and platelet activation using *in vivo* and *in vitro* models.**

PEREIRA, L.D.; GONZAGA, B.M.D.S.; COELHO, L.L.; GARZONI, L.R..
FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRAZIL.

Chagas disease (CD),caused by the protozoan *Trypanosoma cruzi*,represents a serious public health issue, especially in Latin America. The infection is associated with changes in microcirculation and platelet function, both of which contribute to disease progression. Our group has shown that acute *T. cruzi* infection induces functional capillary rarefaction, microvascular inflammation, platelet-leukocyte aggregates on the endothelium, and endothelial dysfunction in the brains of mice. Although treatment with benznidazole or nifurtimox is effective, their adverse effects,particularly without proper medical supervision,often lead to discontinuation. In this context, exploring complementary therapeutic alternatives is essential. Nitric oxide (NO), produced from L-arginine (L-ARG), regulates endothelial function and platelet activation, making it a promising therapeutic target. This study investigated the effects of L-ARG supplementation on cerebral microcirculation and platelet function, in vitro and in vivo. bEnd.3 endothelial cells and Swiss Webster mice infected with the Y strain of *T. cruzi* were used. Parasitemia and body weight were monitored. Cerebral microcirculation was assessed via intravital microscopy, and platelet activation was evaluated using flow cytometry and Western blotting. In vitro assays were also conducted with trypomastigote and amastigote forms of the parasite. L-ARG reduced parasitemia and P-selectin expression in isolated platelets but did not prevent cerebral microvascular changes. Greater thrombin sensitivity was observed in platelets from infected animals. L-ARG was effective against trypomastigotes but had no effect on intracellular amastigotes. These findings suggest that L-ARG may represent a complementary therapeutic approach in CD, acting on both the parasite and platelet activation.

Supported by: FIOTEC
Keywords: Doença de Chagas;L-arginina;Microcirculação.

HP – 083 - **Long-Term Monitoring of Canine Visceral Leishmaniasis Treatment: Clinical, Histopathological, and Parasitic Load Assessment**

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UFSC, FLORIANÓPOLIS - SC - BRAZIL.

Canine visceral leishmaniasis (CVL) caused by *Leishmania infantum* in Brazil has domestic dogs as the main reservoirs in the urban transmission cycle. Miltefosine (MF) has been used as a unique therapeutic alternative for CVL, but studies on its efficacy remain scarce. In this study, we monitored 26 naturally infected dogs from Florianópolis (SC, Brazil) for over 12 months following treatment, as follows: the day before initiating MF treatment (T0), immediately after (T1), six (T2), and 12 months (T3) post-treatment. Three dogs (12%) were exclusively treated with MF, whereas 23 dogs (88%) received a combination of MF with allopurinol, domperidone, and/or immunotherapy. Along with clinical evaluation, histopathological assessment of the inflammatory response in the skin, as well as determination of parasite load by qPCR, were carried out. Molecular and parasitological analysis confirmed the presence of *L. infantum* in the skin of all dogs at T0. Clinical staging revealed significant differences between T0 and T1 ($p=0.0015$), T2 ($p=0.0027$), and T3 ($p=0.0006$), confirming a favorable clinical evolution, except for two dogs (8%) that showed clinical worsening at T3. A significant reduction in parasite load was observed between T0-T1 ($p=0.0065$), T0-T2 ($p=0.00006647$), and T0-T3 ($p=0.0017$), indicating therapeutic efficacy, although no differences were detected between T1-T2 ($p=0.5959$) and T2-T3 ($p=0.9634$), suggesting stabilisation of parasite load. Dogs exclusively treated with MF showed increased parasite load and worsening of clinical signs at T2, suggesting a reduced efficacy of monotherapy. A reduction in the intensity of the cutaneous inflammatory response over the post-treatment period was observed. These results reveal the effectiveness of combined MF-based therapies for CVL, reducing the cutaneous parasite load and inflammatory response and promoting the improvement of clinical signs in naturally infected dogs.

Supported by: FAPESC (2024TR002350), CNPq (445196/2024-6)
Keywords: canine visceral leishmaniasis;zoonosis;public health.

HP – 084 - **Evaluation of the effect of carvedilol on fibrosis in cardiac spheroids/organoids during Trypanosoma cruzi infection**

DA SILVA, D.M.; COELHO, L.L.; VIANNA, M.M.; SEYDEL, C.M.; GARZONI, L.R..
FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRAZIL.

Chronic Chagasic Cardiomyopathy (CCC) is the main complication of Chagas disease (CD), characterized by progressive fibrosis and cardiac hypertrophy, causing electrical and contractile dysfunction. Effective therapies, especially against fibrosis, remain limited. Carvedilol (Carv) is a β-blocker with vasodilatory, anti-inflammatory, antioxidant, and cardioprotective properties, used to treat hypertension, heart failure, and CCC symptoms. Studies show Carv reduces fibrosis in liver, lung, and heart tissues, *in vitro* and *in vivo*, making it relevant to CD. We developed a 3D cardiac spheroid/organoid model reproducing fibrosis and hypertrophy induced by *Trypanosoma cruzi* infection, mimicking tissue architecture and microenvironment, useful for testing repositioned antifibrotic drugs. We evaluated Carv's effects on extracellular matrix (ECM) modulation and *T. cruzi* infection. Primary cardiac cells from mouse fetuses were isolated and characterized by Panoptic staining and immunofluorescence for Troponin T, α-SMA, and CD31. Spheroids were assessed for ECM production, cellular composition, and contractility. Carv cytotoxicity was tested with two assays, showing no toxicity. Infection lasted 72 or 144 hours, followed by 72 hours treatment with 10 μM Carv. Cell viability, fibronectin expression, and parasite load were evaluated. Parasite life cycle was assessed in monolayers. Spheroids showed characteristic morphology, structural integrity, and contractility. Infection increased fibronectin, indicating fibrosis; no significant difference was found between untreated and Carv-treated infected groups. A trend of parasite load reduction and decreased trypomastigotes occurred with treatment. The 3D model reproduced CCC fibrotic events, confirming its usefulness. Although Carv did not reduce fibrosis, it may modulate *T. cruzi* infection.

Supported by: CAPES, FIOCRUZ
Keywords: Chagas disease;Three-dimensional culture (3D);Cardiac fibrosis.

HP – 085 - **Prediction in silico of epitopes and characterization of ISC3, ISC6, and TSC5 proteins of Neospora caninum with CRISPR/Cas9-mediated 3×HA tagging**

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Neospora caninum is an apicomplexan parasite closely related to ***Toxoplasma gondii*** and the causative agent of neosporosis, a disease that leads to neuromuscular disorders in dogs and abortion in cattle, resulting in significant economic losses in livestock. Despite its impact, only a few antigenic targets have been investigated for diagnosis and vaccine development. In this study, we aimed to evaluate the antigenic potential of three candidate proteins (ISC3, ISC6, and TSC5) by predicting T- and B-cell epitopes and experimentally tagging these proteins. Protein sequences were obtained from the ToxoDB database (NcLiv reference genome). Epitope predictions for murine MHC class I and II alleles were performed using NetMHCpan and the Consensus method, while linear B-cell epitopes were predicted using BepiPred. Selection criteria included high binding affinity, immunogenic potential, and conservation among strains. The in silico analysis revealed multiple epitopes with strong predicted affinity to MHC class I and II molecules, as well as surface-exposed and accessible B-cell epitopes, reinforcing their potential immunogenicity. In addition, we employed CRISPR/Cas9-mediated genome editing to generate transgenic ***N. caninum*** parasites expressing ISC3, ISC6, and TSC5 fused to a C-terminal 3×HA tag. This approach enabled the determination of protein size and subcellular localization, contributing to the functional characterization of these proteins. Altogether, our findings highlight ISC3, ISC6, and TSC5 as promising targets for further functional validation and immunobiological studies

Supported by: FAPEMIG APQ-00143-23; CAPES e CNPq
Keywords: Epitope prediction; Neospora caninum;CRISPR/Cas9.

HP – 086 - **Opsonization with antibody acts synergistically with IL-10, increasing susceptibility of murine macrophages to *L. braziliensis* promastigote infection *in vitro***

MARQUES, A.M.D.S.[1]; SOARES, S.A.E.[1]; ARAUJO, J.A.A.[1]; DE VASCONCELOS, B.S.[1]; MENDES, H.K.F.[1]; FERNANDES, F.X.[1]; GOMES, C.M.[2]; DE MATOS, G.G.[1]; DE OLIVEIRA, M.A.P.[1].
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Infection of BALB/c mice with different strains of *L. braziliensis* usually causes a non-ulcerated small lesion that can be self-healing or be maintained as small for several weeks. *In vitro*, murine inflammatory BALB/c macrophages are generally resistant to infection with different *L. braziliensis* strains. Here, we used a field-isolated JCJ8c strain of *L. braziliensis* to evaluate the role of long-term treatment of murine macrophages with IL-10 and antibody opsonization on the susceptibility of macrophages in vitro. Inflammatory thioglycolate-elicited BALB/c macrophages were cultured either with or without IL-10 for 1 to 6 days and then infected with L. braziliensis promastigotes that were opsonized or not with murine anti-leishmania antibodies. Untreated inflammatory macrophages internalize non-opsonized promastigotes; however, they can kill most parasites until 5 days after infection. Treatment with IL-10 decreased IL-12p40 production and increased arginase activity in a time-dependent manner; however, it did not change the ability of macrophages to produce reactive oxygen species and nitric oxide. Additionally, IL-10 treatment decreased the mortality of *L. braziliensis* amastigotes five days after infection, and opsonization of the parasites increased the proliferation of amastigotes, primarily within IL-10-treated macrophages. Our results demonstrated that IL-10 renders murine macrophages susceptible to L. braziliensis, and this susceptibility is increased by antibody opsonization. These data suggest that this environment may be responsible for creating a safe target cell for L. braziliensis infection in the murine model.
Supported by: INCT-IPH, CNPq, CAPES, FAPEG
Keywords: M2 macrophage, ;Leishmania braziliensis;amastigote proliferation.

HP – 087 - **Comparative study between Crithidia-related isolates obtained from the skin and bone marrow of a patient with leishmaniasis**

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A Brazilian case was reported involving a patient diagnosed with visceral leishmaniasis (VL) who presented with a coinfection involving parasites morphologically related to Leishmania, but molecularly identified as belonging to the genus Crithidia. Protozoa of the genus Crithidia are classified as monoxenous because they infect only invertebrate hosts. However, cases showing that they can survive in coinfection with HIV and Leishmania spp. have been reported. Recently, Crithidia sp. LVH60A was identified in cultures from 48 clinical isolates of patients with VL and in 3 bone marrow samples from patients coinfected with L. infantum. These parasites were isolated from patients who showed resistance to leishmaniasis treatment, highlighting the importance of studying how these isolates interact with human immune cells, such as neutrophils. This work aims to analyze the interaction of Crithidia sp. isolates LVH60A (from bone marrow) and LVH60ac1 (from skin) with the complement system and neutrophils. Both isolates were cultured in Schneider medium with 5% fetal bovine serum at 26°C, and parasites from the logarithmic (24/48 h) and stationary (6/7 days) growth phases were compared. Incubation with fresh human serum for 90 min showed a titration of 32 for log and stationary LVH60ac1, and 8 and 16 for log and stationary LVH60A, as measured by resazurin. Neutrophils purified from the blood of healthy donors were incubated with the parasites (1 neutrophil: 1 Crithidia), and neutrophil extracellular traps (NETs) were measured after 90 min using picogreen. Our results showed that stationary LVH60ac1 induced significantly more NETs than the log phase parasites. Both log and stationary phase LVH60A induced NETs equally. We also tested whether these parasites caused ROS production upon interacting with neutrophils using Amplex Red. Our results showed no difference in ROS production when both growth phases of both parasites interacted with neutrophils.
Supported by: FAPERJ, CNPQ
Keywords: Neutrophil extracellular traps;Visceral leishmaniasis;Crithidia sp;.

HP – 088 - **Macrophages infected with *T. gondii* show increased arginase I activity, however, urea production was not detected in the supernatant by colorimetric assay**

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Macrophages are essential immune cells responsible for maintaining and defending the body. In tissues, these cells are found in a non-activated state (M0) but can be polarized into a pro-inflammatory phenotype (M1), with enhanced phagocytic and microbicidal activity, or an anti-inflammatory phenotype (M2). M2 macrophages contribute to tissue repair, homeostasis, and inflammation regulation. They express high levels of arginase 1 (ARG1), which hydrolyzes L-arginine into urea and ornithine. *Toxoplasma gondii*, the parasite that causes toxoplasmosis, infects M0 macrophages and shifts their profile toward M2. This study aimed to detect urea in macrophage culture supernatants using the method of Jung et al. (1975), modified by Zawada (2009), and to evaluate ARG1 activity in macrophages infected or not with *T. gondii*. RAW 264.7 macrophages were seeded and stimulated or not with IL-4 and 8-Bromoadenosine-cAMP. M0 and M2 macrophages were infected with *T. gondii*. After 24 h, urea in culture supernatants was analyzed by a colorimetric assay using a solution with o-phthalaldehyde and primaquine bisphosphate in acid solution, and compared to a standard urea curve. ARG1 activity was measured in cell lysates from infected or uninfected M0 and M2 macrophages. Urea could not be consistently detected in the supernatants of M0 or M2 macrophages, infected or not, using the modified Jung method. This may reflect low sensitivity of the technique, as ARG1 activity was detected in M2 macrophages but not in M0. As expected, M0 macrophages infected with *T. gondii* showed increased ARG1 activity compared to uninfected M0 cells, indicating a shift to an M2-like profile. However, urea production was likely too low for detection by this method.

Keywords: Toxoplasma gondii;Macrophage ;urea.

HP – 089 - **Pharmacological inhibition of *Toxoplasma gondii* ATR-like kinase impairs parasite replication**

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Toxoplasma gondii rapidly replicates during the acute phase of toxoplasmosis. This rapid replication could lead to genome instability due to the collapse of replication forks, requiring effective repair for cell cycle progression. ATR (ATM and Rad3-related kinase), is a master kinase involved in DNA repair and cell cycle regulation. Given its importance, our goal is to gain a deeper understanding of the DNA damage repair (DDR) system in *T. gondii* by investigating ATR. In this study, we infected HFF hTERT cells with parasites of the RH ΔHXGPRT strain to examine the functional role of TgATR using specific inhibitors (VE-821 and its analogue VE-822). We evaluated critical biological processes in the parasite such as growth and replication. Both inhibitors have been demonstrated to have an antiproliferative effect with CC50 50μM; IC50 1 μM and CC50 1.7 μM; IC50 0,125 μM, respectively. VE-821 at 6 μM reduced the tachyzoite replication rate 2.3 fold as total number of tachyzoites per parasitophporous vacuoles (PV). Moreover, we observed 28.9% PV with 1 tachyzoite in treated *T. gondii* and 4.55% PV with 1 tachyzoite in untreated *T. gondii*. Similarly, VE-822 (0.25 μM) decreased replication 2-fold, with a striking accumulation of vacuoles containing a single parasite (78% vs. 33.6% in untreated). These results highlight a pronounced replication defect upon ATR inhibition, linking a genome instability process in the fast replicative tachyzoite.

Supported by: INCT/ CNPq 406572/2022-4. CAPES Cód de financiamento 001. ANPCYT PICT 2021 331
Keywords: ATR inhibitor ;DNA Damage Repair System ;Toxoplasma gondii .

HP – 090 - **Dissecting the Function of NLRP12 and miR-294 in Macrophage Responses to Leishmania amazonensis**

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Leishmaniasis is a neglected tropical disease caused by protozoa of the genus *Leishmania*, transmitted to humans by sand flies. It can present in cutaneous, mucocutaneous, or visceral forms, depending on the parasite species and the host immune response. *Leishmania amazonensis*, relevant in Brazil, causes cutaneous leishmaniasis and primarily infects macrophages, surviving within the phagolysosome and modulating inflammatory pathways, such as inflammasome formation. One immune evasion mechanism involves microRNAs (miRNAs), small endogenous RNAs that inhibit gene expression. In mice, miR-294 is transcribed from an intronic region of the Nlrp12 gene, which negatively regulates inflammasome activation and inflammation. This study aimed to investigate the role of NLRP12 and miR-294 in macrophage responses to *L. amazonensis* infection. In BALB/c mice, which are susceptible to infection, expression of NLRP12, premiR-294, and miR-294 increased at 4 h post-infection, followed by a decrease in NLRP12 and premiR-294 at 24 h, with no modulation by LPS. IL-1β, IL-18, and NLRP3 were not modulated by infection, but IL-1β and NLRP3 increased after LPS stimulation. In C57BL/6 mice, which are resistant to infection, miR-294 expression increased at 24 h, and IL-1β increased only in response to LPS. Inhibition of miR-294 in infected C57BL/6 macrophages increased NLRP12 and IL-1β expression and reduced the frequency of infected macrophages and the number of amastigotes per cell (MFI) at 4 h. Additionally, silencing CEBP/β (a transcription factor for NLRP12) and RelA (a TLR-activated NF-κB subunit) also reduced infection at 4 h and 24 h, suggesting that regulation of NLRP12 by miR-294, CEBP/β, and RelA influences the host response.
Supported by: Fapesp - código do processo 2025/04633-5 , CAPES - código de apoio 001
Keywords: Leishmaniasis;microRNA;macrophages.

HP – 091 - **Evaluation of CD200 Expression in Dendritic Cell Populations from Draining Popliteal Lymph Nodes of *Leishmania* (*Leishmania*) *amazonensis*-Infected C57BL/6 Mice**

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Leishmania (*Leishmania*) *amazonensis* is one of the etiological agents of cutaneous leishmaniasis, an endemic disease in tropical and subtropical regions, often associated with diffuse and more severe clinical forms. Dendritic cells (DC) play a central role in the immune response by recognizing the pathogen and presenting antigens to T cells, thereby directly influencing disease progression. Although it is known that *Leishmania* can manipulate DC function, the underlying mechanisms remain poorly understood. The inhibitory glycoprotein CD200 has been previously described as upregulated in infected macrophages, favoring parasite survival; however, its role in DC is still unknown. This study evaluated CD200 expression in DCs from draining popliteal lymph nodes of *L.* (*L.*) *amazonensis*-infected C57BL/6 mice. Female C57BL/6 mice (n = 4) were subcutaneously infected in the left hind footpad with 1×10^6 stationary-phase promastigotes for six weeks. The draining popliteal lymph node was analyzed by flow cytometry. DC were defined as $CD45^+$ $F4/80^-$ $MERTK^-$ $CD206^-$ $CD11c^+$ MHC II^+ and compared with non-infected control. Statistical analyses were performed using one-way ANOVA, two-way ANOVA, and Tukey's test (GraphPad Prism). The DC population within viable $CD45^+$ cells was significantly higher in infected animals (>1000) compared to controls (>10). Two DC subsets were identified: MHC II^high (migratory, migDC) and MHC II^intermediate (resident, resDC). In infected mice, the migratory subset predominated (80% migDC vs. 20% resDC), whereas in non-infected controls the opposite was observed (30% and 70%, respectively). More than 80% of MHC II^high DC expressed CD200 regardless of condition, but the expression intensity was higher in infected animals (MFI ~4000) compared to controls (MFI ~1500). In MHC II^int DCs, CD200 expression was lower (30–60%), with a modest increase in infected mice (MFI ~1300 vs. ~500). In conclusion, *L.* (*L.*) *amazonensis* infection increased the frequency of DC in draining lymph nodes, with a predominance of MHC II^high (migDC) displaying high CD200 expression, suggesting a potential role of this pathway in immune modulation and parasite evasion. However, these results should be interpreted with caution due to the small sample size and the absence of additional markers for lymphocytes, Langerhans cells, and specific DC subpopulations. Future studies with larger cohorts and broader panels are required to confirm these findings.
Supported by: FAPESP 2025/02593-6
Keywords: Leishmaniasis;Dendritic cells;Lymph node.

HP – 092 - **Advances in a Three-Dimensional Cardiac Model for the Study of *Trypanosoma cruzi*-Induced Fibrosis: Evaluation of Carvedilol's Effect**

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INSTITUTO OSWALDO CRUZ - FIOCRUZ, RIO DE JANEIRO - RJ - BRAZIL.

Chronic Chagasic cardiomyopathy (CCC) is the main complication of Chagas disease (CD), characterized by cardiac hypertrophy and progressive fibrosis. Effective therapies against fibrosis remain limited. Carvedilol (Carv) is a β-blocker with vasodilatory, anti-inflammatory, antioxidant, and cardioprotective properties, used in the treatment of hypertension, heart failure, and CCC symptoms. Studies have shown that Carv reduces fibrosis in liver, lung, and heart tissues, both *in vitro* and *in vivo*; however, its effect on fibrosis in CD remains unknown. We developed a 3D model of cardiac spheroids/organoids that reproduces fibrosis and hypertrophy induced by *Trypanosoma cruzi* infection, mimicking tissue architecture and the microenvironment, and providing a platform to test antifibrotic drugs. We evaluated the effects of Carv on extracellular matrix (ECM) modulation and *T. cruzi* infection. Primary cardiac cells from mouse fetuses were isolated and characterized using panoptic staining and immunofluorescence for troponin T. The spheroids/organoids were assessed for ECM production, cellular composition, and contractility. Carv cytotoxicity was tested in two assays, showing no cytotoxic effects. The infection lasted 144 h, followed by 72 h of treatment with 10 µM Carv. Cell viability, fibronectin expression, α-SMA, and parasite load were evaluated. Infection increased fibronectin expression, indicating fibrosis, whereas Carv treatment significantly reduced this effect, suggesting an antifibrotic action. α-SMA expression decreased in both the untreated and treated infected groups, likely due to cytoskeletal disruption induced by *T. cruzi*. A trend of reduced parasite load was observed with treatment. The 3D model reproduced the fibrotic events characteristic of CCC and enabled the first demonstration of carvedilol's antifibrotic effect in the context of CD, reinforcing its value as an experimental tool for studying pathological mechanisms and therapeutic interventions.

Supported by: CAPES
Keywords: Chagas disease;three-dimensional cell culture;cardiac fibrosis.

HP – 093 - **Modular DNA vaccine design to improve antigen delivery in Trypanosoma cruzi vaccine candidates**

CARVALHO, A.M.D.S.[1]; ROSA, D.F.[1]; MANGABEIRA, K.S.D.S.[1]; BOAVA, A.D.[1]; SILVA, J.L.B.E.[1]; DE MELO, F.D.S.M.[1]; SILVA, K.D.S.[1]; FERCOQ, F.[2]; MOTTA, F.N.[1]; LONGO, J.P.F.[1]; CHARNEAU, S.O.[1]; DE ARAÚJO, C.N.[1]; DE SANTANA, J.M.[1]; MARTIN, C.[2]; BASTOS, I.M.D.[1].
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Controlling Chagas disease is a challenge, and therefore efforts should focus on developing new strategies. In this context, nucleic acids represent excellent opportunities in vaccine development, as they are versatile and can be designed to induce a specific, robust, and long-lasting immune response. Thus, the objective of this study is to develop a DNA vaccine platform. Initially, a proof of concept was carried out using the optimized optV vector cloned with the chimeric protein luciferase and mNeongreen (LucNeoG). In vitro characterization demonstrated expression of the LucNeoG antigen. In vivo analysis, it showed high expression 24 hours after inoculation. Subsequently, female Balb/c mice were immunized with optV-LucNeoG, and the vaccine delivery routes were analyzed. The results showed that the intramuscular route was more effective in producing anti-LucNeoG IgG antibodies. Following the proof of concept, the method was validated using an antigen previously employed as a vaccine formulation against *T. cruzi* the 80 kDa prolyl oligopeptidase. The antigen was cloned into the optV vector, and the cloning was confirmed by Sanger sequencing. Subsequently, immunization was performed with 25, 50, or 100 µg of optV-POPTc80. Immunization with optV-POPTc80 was able to stimulate CD4+, CD8+ T cells, and the production of anti-POPTc80 IgG antibodies. Mice challenged with trypomastigotes showed a reduction in parasitemia during the acute phase. Immunization with optV-POPTc80 reduced the concentration of pro-inflammatory cytokines in splenocytes of vaccinated mice. Histological analysis of the hearts of immunized animals showed a reduction in the inflammatory process. The results suggest that DNA immunization constitutes a promising platform for the search for new targets for vaccine development and that further research is needed on optimization methods to enhance vector immunogenicity and stability.

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Keywords: Chagas disease;Vaccine Platform;Nucleic Acid-Based Vaccines.

HP – 094 - **Integration of Synchrotron Techniques for the Analysis of Cellular Ultrastructures in Acanthamoeba castellanii Induced by the Niemeyer Virus**

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Giant viruses, such as those belonging to the *Mimivirus* genus, have attracted considerable attention in virology due to their large capsids, extensive genomes, and complex replication cycles, which challenge traditional paradigms of the virosphere. Among them, the Niemeyer virus (NYMV), which infects *Acanthamoeba castellanii*, induces profound intracellular alterations, particularly through the formation of viral factories (VF), dense structures where replication occurs. In this study, we employed a multimodal approach integrating advanced synchrotron techniques to investigate in detail the effects of NYMV infection on host cell organization and biochemical properties. Analyses were conducted using Cryo Soft X-ray Tomography (Cryo-SXT) at the ALBA synchrotron (Spain) and Ptychographic X-ray Computed Tomography (PXCT) at the Cateretê beamline of SIRIUS (LNLS, Brazil), enabling nanometric-scale three-dimensional reconstructions. In parallel, infrared microspectroscopy at the Imbuia beamline (SIRIUS, LNLS) was used to assess biochemical signatures associated with infection, complemented by Atomic Force Microscopy (AFM), which allowed the evaluation of cell topography, organelle and VF localization, as well as nanomechanical properties. The integration of these methodologies provided a comprehensive view of the structural and biochemical remodeling promoted by NYMV, enabling the observation of viral assembly and the inference of related functional aspects. In conclusion, this study highlights the potential of combining synchrotron-based imaging, spectroscopy, and nanometrology techniques to achieve a three-dimensional and multidimensional understanding of giant virus–host interactions, contributing to a deeper knowledge of the biology of complex viruses under conditions close to their native state.
Supported by: 88887.901569/2023-00
Keywords: Giant viruses;Synchrotron imaging;Viral factories.

HP – 095 - **Development of a VLP-based vaccine targeting the thimet oligopeptidase of *Trypanosoma cruzi***

BOAVA, A.D.; SILVA, K.D.S.; CARVALHO, A.M.D.S.; ROSA, D.F.; SILVA, J.L.B.E.; DE MELO, F.D.S.M.; TORRES, F.A.G.; DE ARAÚJO, C.N.; DE SANTANA, J.M.; BASTOS, I.M.D..
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Chagas disease (CD), caused by *Trypanosoma cruzi*, remains a major public health challenge due to limited therapeutic options and the absence of a vaccine. Among prophylactic strategies, proteases have emerged as relevant targets, including thimet oligopeptidase (TOPTc), a zinc-dependent metalloprotease of the M3 family, found both in the secretome and on the surface of the parasite at different stages of its life cycle. In this context, the present study aimed to develop a chimeric vaccine based on virus-like particles (VLPs). Immunogenic regions of TOPTc were fused to the hepatitis B surface antigen (HBsAg). High-affinity B- and T-cell epitopes were predicted using computational tools such as NetMHCpan-4.1, NetMHCIIpan-4.1, and BepiPred-2.0, ensuring minimal homology with host proteins and conservation across distinct *T. cruzi* strains. The selected epitopes were codon-optimized and genetically fused to HBsAg to enable VLP display. The chimeric TOPTc-HBsAg construct was cloned into the pPink-HC plasmid and expressed in *Komagataella phaffii*. Protein expression was confirmed by Western blot. Following purification by ultracentrifugation in CsCl gradients (1.1 g/mL and 1.3 g/mL), particle size was determined by dynamic light scattering (DLS; 28.01 ± 8.795 nm), and structural conformation was validated by transmission electron microscopy (TEM). This VLP-based platform represents a scalable and highly immunogenic delivery system, supporting its potential for future preclinical evaluation against CD.
Supported by: This work was supported by grants awarded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, 88881.711954/2022-01 CAPES-COFECUB); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior Finance code 001, the CNPq (316892/2023-8), and Fundação de Apoio à Pesquisa do Distrito Federal (FAP-DF, Grants 00193-00002136/2023-01 and 00193-00000833/2021-57).
Keywords: Virus-like particles;thimet oligopeptidase;Chagas disease.

HP – 096 - **Molecular characterization of the serine peptidase inhibitor 2 of *Trypanosoma rangeli* (TrgISP2) for the development of differential diagnostic methods for Chagas disease.**

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Trypanosoma rangeli is a hemoflagellate protozoan that shares vectors and hosts with *Trypanosoma cruzi*, the etiological agent of Chagas disease. Although it is not pathogenic to humans, its presence can hinder differential diagnoses due to the antigenic similarity between the species. The serine peptidase inhibitor 2 (ISP2) has already been characterized in *T. cruzi* as a factor associated with immune evasion and a potential molecular marker. However, little is known about ISP2 from *T. rangeli* (TrgISP2) and its role in parasite–host interaction. This study aims to molecularly characterize the gene encoding TrgISP2 and its recombinant protein, employing PCR, cloning, protein expression, and enzymatic activity assays. The motivation for this research stems from the previous detection of *T. rangeli* in samples analyzed by the research group, which prompted the initiation of experimental tests for amplification and cloning of the target gene. Structural and functional comparisons between TrgISP2 and TcISP2 may indicate specific markers for the differential diagnosis of Chagas disease, contributing to greater accuracy in epidemiological studies and the development of molecular tools. The expected results include confirmation of the presence and expression of the TrgISP2 gene, as well as initial data on the functionality of the recombinant protein, thus establishing a foundation for future research and advances in public health.

Supported by: FAPESP E CNPQ
Keywords: Trypanosoma rangeli;ISP2;molecular diagnosis.

HP – 097 - **In vitro assessment of the trypanocidal activity of a molecule identified by an artificial intelligence approach**

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Chagas disease, caused by the protozoan *Trypanosoma cruzi*, is a serious public health problem affecting millions of people. Currently, the only drugs available for treatment are benznidazole and nifurtimox. Despite their widespread use, these drugs show limited efficacy, particularly in the chronic stages of infection, and are associated with side effects that contribute to poor treatment adherence. Given these limitations, there is an urgent need to search for new bioactive molecules, both synthetic and natural, with higher selectivity, lower toxicity, and efficacy across different phases of infection, offering safer and more effective alternatives. The aim of this study is to further evaluate the amastigocidal effect of compounds generated by artificial intelligence. Five compounds that demonstrated efficacy against *T. cruzi* epimastigotes (Tulahuen and CL-Brener strains) were prioritized and subsequently investigated against the intracellular replicative form of the parasite. L6 cells were cultured in 96-well plates and infected with *T. cruzi* trypomastigotes at a 30:1 ratio for 24 h. Non-internalized parasites were removed, and cells were treated with the compound (100–0.007 µM) for 72 h or longer. Analyses were performed by panoptic staining or luminescence. The compound was further assessed using a wash-out protocol, with readings taken at 5 and 15 days post-treatment. Against the Y strain, the IC_{50} value was 40 nM. In the wash-out assay, LC-6 inhibited 100% of the infection up to 360 h, with parasite regrowth observed only at concentrations above 62.5 nM. Scanning electron microscopy of trypomastigote forms treated with LC-6 revealed drastic alterations in the parasites. In conclusion, the compound discovered through artificial intelligence exhibits low toxicity and high trypanocidal activity, with a prolonged effect at the nanomolar range, indicating its potential as a candidate for new therapies against Chagas disease.

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Keywords: bioluminescence;treatment;cell assay.

HP – 098 - **PEP01, a nitroimidazole inhibitor of cysteine protease B (CPB) from *Leishmania infantum*: design, synthesis, and evaluation through *in silico, in vitro*, and *in vivo* approaches**

MELA, M.F.D.S.[1]; DE LIMA, J.S.[1]; VELÁSQUEZ, A.M.A.[2]; ROMANO, J.[1]; PRATES, J.L.B.[1]; COELHO, N.C.S.C.[1]; YAGI, A.C.L.[1]; CILLI, E.M.[3]; DOS SANTOS, J.L.[1]; GRAMINHA, M.A.S.[1].
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Leishmaniasis remains a major Neglected Tropical Diseases and current treatments are limited by toxicity and emerging resistance, highlighting the urgent need for safer and more effective drugs. In this study, we designed, synthesized, and characterized PEP-01, a nitroimidazole derivative targeting cysteine protease (CPB), an key enzyme associated with parasite virulence. PEP-01 displayed potent CPB inhibition (IC_{50CPB} = 4 µM), high anti-amastigote activity (IC_{50AMA} = 3.9 µM) and low cytotoxicity (CC_{50} = 300 µM), resulting in a high selectivity index (SI = 77). In silico ADME (Absorption, Distribution, Metabolism, and Excretion) prediction indicated favorable oral bioavailability, with good solubility and moderate lipolicity. In toxicity, using *Galleria mellonella* larvae, PEP-01 was well tolerated at dose up to 77 mg/kg/day. In a murine model of *L. infantum*, infection, oral administration of PEP-01 (0.77 mg/kg/day for 7 days) reduced parasite burden by ~40% in the liver and ~50% in the spleen. Biochemical analyses suggested mild hepatic alterations without evidence of irreversible damage. Altogether, these findings support CPB as a valid drug target and demonstrate that PEP-01 is a promising candidate for the development of safe, selective, and orally available antileishmanial agents.
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Keywords: Drug discovery;Leishmaniasis;Cysteine protease B.

HP – 099 - **Investigating lpg2 Function in Dendritic Cell Migration during *Leishmania infantum* Infection**

BERNARDES, C.P.O.S.; DE AZEVEDO, B.H.; NERY, G.L.; SANTOS, F.H.D.J.; PAIXÃO, A.R.; GOICOCHEA, A.M.C.; LUZ, Y.D.S.; FARIAS, L.P.; BRODSKYN, C.; VERAS, P.S.T.; BORGES, V.; FULLAM, J.P.B.D.M..
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Leishmaniasis is a zoonotic disease caused by Leishmania protozoa and can manifest in various clinical forms. The dissemination of the cells containing *L. infantum* are crucial for the parasite survival and establishment of the disease. Studies suggest that Leishmania lipophosphoglycan (LPG) plays a key role in host cell interactions; however, its relationship with dendritic cells (DCs) remains unclear. The objective of this work is to evaluate the role of lpg2 in migration of human DCs infected with *L. infantum*. To investigate these interactions, human DCs were infected with wild-type *L. infantum*, Δlpg2, or Δlpg2 + lpg2 and analyzed using a Transwell system. Immunostaining was performed with antibodies against pFAK and pPaxillin to assess adhesion complex formation, Talin and Vinculin to evaluate podosome formation, and Rac1, Cdc42, Gelsolin, RhoA, and phalloidin to examine actin polymerization via confocal microscopy. CCR7 expression in DCs infected with different Leishmania strains was assessed by flow cytometry. Additionally, ERK1/2 pathway activation was evaluated by analyzing ERK1/2 expression using western blotting.The results showed that DCs infected with L. infantum Δlpg2 exhibited reduced migration compared to those infected with wild-type *L. infantum* or Δlpg2 + lpg2. This data was associated with the reduction in the formation of adhesion complexes, podosome and actin polymerization. Additionally, lower CCR7 and ERK1/2 expression was observed in DCs infected by *L. infantum* Δlpg2 compared to cells infected with the other strains. The data presented in this study suggest that L. infantum LPG modulates positively migration of DCs by enhancing adhesion complexes formation, podosome assembly, actin dynamics, as well as CCR7 expression and ERK1/2 pathway activation. Further experiments are needed to better elucidate the mechanisms by which LPG regulates DCs infected with *L.infantum*.
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Keywords: Leishmania;Dendritic Cells;Migration.

HP – 100 - **Exploring Orc1b's Contribution to DNA Replication Dynamics in *T. cruzi***

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Proper maintenance of the genetic material requires strict control of DNA replication. This process is mediated by the pre-replication complex (PRC), which in most eukaryotes is composed of the Orc complex and the proteins Cdc6 and Cdt1, acting together to allow the action of the MCM complex. In Trypanosoma cruzi (T. cruzi), the etiological agent of Chagas disease, the composition of Orc is still under debate, with a protein called Orc1Cdc6 having been described. An additional factor, Orc1b, has been identified as an interactor of Orc1Cdc6 only during the S phase of the cell cycle in Trypanosoma brucei. Our group has also previously demonstrated enrichment of replication origins in regions of rapidly evolving multigenic families. To investigate the role of Orc1b, we performed ChIP-seq assays for Orc1Cdc6 and Orc1b, which revealed colocalization of the peaks of these proteins and association of Orc1b with active replication origins. To further this analysis, we carried out CUT&RUN for the MCM7 protein, aiming to identify its binding sites and assess overlap with Orc1b at active origins. Additionally, we are generating a T. cruzi CL Brener lineage expressing Orc1b with a MYC tag, which will allow us to evaluate its dynamics throughout the cell cycle and perform co-localization experiments by immunofluorescence. These results will help elucidate the role of Orc1b and provide an integrated view of DNA replication dynamics in T. cruzi.
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Keywords: Trypanosoma cruzi;DNA replication;Orc1b.

HP – 101 - **EVALUATION OF THE ANTIMALARIAL EFFECT OF GERANYLGERANYLACETONE AND ITS ACTION ON ISOPRENOID BIOSYNTHESIS**

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Malaria is a disease caused by the protozoan of the genus *Plasmodium* and represents a serious public health problem. The resistance of *Plasmodium* to antimalarial drugs has become a challenge, justifying the search for new compounds and effective pharmaceutical combinations. One of the most severe complications of malaria is its cerebral phase, a neurological syndrome with the risk of coma, several permanent sequelae, and potentially fatal outcomes. Therefore, our research group has previously demonstrated that several natural terpenes exhibit antimalarial effects *in vitro* and *in vivo*, and many of these terpenes affect the isoprenoid metabolism of the parasite and may improve the efficacy of antimalarial drugs. Based on this, we evaluated the *in vitro* and *in vivo* antimalarial potential of geranylgeranylacetone (GGA) in *Plasmodium falciparum* and *Plasmodium berghei*. Our results showed that GGA has an *in vitro* antiplasmodial effect against the 3D7 strain with an IC_{50} of 14.95 ± 4.81 µM and also exhibited low toxicity in HEP-G2 cells (human hepatocarcinoma) with a CC_{50} of 352.57 ± 33.71 µM and an SI of 23.58. Furthermore, it inhibited protein isoprenylation in *Plasmodium falciparum* in assays with radioactive FOH. However, in *in vivo* assays, GGA did not show efficacy in reducing parasitemia in mice. Nonetheless, GGA demonstrated good efficacy in the treatment of murine cerebral malaria in C57BL/6 mice infected with PbANKA, with a survival rate of 70% in the GGA-treated group compared to 30% in the control group. It is expected that the results obtained in this research may contribute to the development of new therapeutic strategies for cerebral malaria and further studies regarding its mechanism of action.
Supported by:
Keywords: cerebral malaria;isoprenoids;geranylgeranylacetone.

HP – 102 - **Species Turnover of Ciliates (Ciliophora) Along an Altitudinal Gradient in the Colotepec River, Oaxaca, Mexico**

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Abstract: The component of diversity that measures species turnover between different communities (beta diversity) has been key to understanding biodiversity patterns in tropical regions such as Mexico. Although studies using beta diversity as a reference for understanding diversity in different taxa exist, its application in ciliate studies remains limited. Ciliates are unicellular organisms that play a key role in the microbial loop. They are widely distributed in various aquatic environments, but studies on beta diversity in river systems are scarce.

This research evaluates both the spatial and temporal turnover of ciliate species along an altitudinal gradient in the Colotepec River, Oaxaca, Mexico. The results include the diversity recorded in two samplings (March and July 2025) at four sampling sites along the river. Two samples were collected at each site, one from organic matter and another from rock-scraping, the ciliates were identified using bright-field and differential interference contrast light microscopy, employing both temporary preparations for in vivo observations and photomicrography registration, as well as permanent preparations for more precise taxonomic identification.

To date, 56 ciliate taxa have been identified. Preliminary results indicate a certain degree of turnover in the ciliate species composition both between different altitudinal sites and between seasons. This turnover could be attributed to a combined influence of environmental, spatial, and temporal factors. This study represents the first faunal inventory of free-living ciliates in the Colotepec River and aims to contribute with new data on ciliate diversity in the state of Oaxaca and in Mexico, as well as to highlight the importance of considering microorganisms in beta diversity studies.

HP – 103 - **Diversity and turnover of interstitial ciliates on two beaches in Mexico**

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The phylum Ciliophora, which has about 8,000 species, is grouped in the clade Alveolata and is considered a monophyletic group. Ciliates can be found in different bodies of water anywhere in the world, as well as in sediments, and can be free-living or symbiotic with different organisms. Those that inhabit the spaces between sediments are known as interstitial, a microfauna present in both freshwater and marine sediments, with the latter exhibiting greater faunal diversity. Currently, 1,000 species of interstitial ciliates have been described for both environments. Unlike the study of interstitial ciliates in Mexico has been scarce. The objective of this study is to compare the variation in the species composition of interstitial ciliates in relation to their geographical distribution and seasonality on two beaches on the Mexican Pacific coast. Sampling was carried out in March, April, and July (2025), collecting four sediment cores for one of the beaches and two cores for the other. The samples were processed using the Uhlig method and observed in vivo. Microphotographs were obtained for subsequent identification, and some samples were fixed for further techniques. Twelve taxa have been identified so far, of which turnover has been noted between both study sites since only 3 of the 12 nominal taxa are shared. Due to the diversity of ciliates and the few taxonomic studies conducted on these organisms, it is necessary to continue the line of research focusing on this group of organisms.