TB – 004 - **Synthetic 4-aminoquinazoline derivatives with activity in vitro against amitochondriate *Trichomonas vaginalis***

REZENDE, A.L.L.; DOS SANTOS, W.T.; LADVOCAT, A.C.C.D.P.; BARBOSA, M.L.D.C.; JAEGER, L.H..
UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA - MG - BRAZIL.

Trichomonas vaginalis is an amitochondriate facultative anaerobic protozoan which causes the sexually transmitted infection trichomoniasis. Nitroimidazole drugs, including metronidazole (MTZ), are the only therapeutic option to treat the infection. However, clinical cases refractory to this treatment have emerged in recent years. Quinazoline derivatives have shown several in vitro pharmacological activities, including antiprotozoal activity. Thus, aminoquinazoline derivatives were evaluated in vitro for their efficacy against an MTZ-resistant *T. vaginalis* strain and their cytotoxicity against human adenocarcinoma cells. Eleven synthetic aminoquinazoline derivatives were analyzed by HPLC for purity confirmation. The effect of these compounds was evaluated on the MTZ-resistant CDC085 strain of *T. vaginalis*. Cell viability of parasite was assessed after the addition of Trypan Blue and the inhibitory concentration of 50% (IC_{50}) was subsequently determined. Cytotoxicity against HeLa lineage was evaluated by the MTT assay and the cytotoxic concentration of 50% (CC_{50}) was determined. The selectivity index (SI) was calculated. Analytical analyses confirmed the purity of the previously synthesized compounds. Five aminoquinazoline derivatives showed in vitro anti-*T. vaginalis* activity, with $IC_{50} < 20$ µM, and of these, two showed outstanding potencies: MLBS11 and MLBS42 with IC_{50} of 5.656 and 6.568 µM, respectively. Overall, the compounds were not toxic to human cells, with the exception of MLBS49 ($CC_{50} < 12.5$ µM). Particularly, the compound MLBS11 was highly selective to parasites (SI = 16.37). Aminoquinazoline derivatives are promising candidates to follow preclinical in vitro and in vivo tests in the treatment of refractory trichomoniasis. Further studies are needed to evaluate the mechanism of action of the compounds against MTZ-resistant *T. vaginalis*.

Supported by: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, N. 001) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, APQ-00149/2023).
Keywords: Analytical characterization;anti-Trichomonas vaginalis activity;Cytotoxicity.

TB – 005 - **Culture supernatants from macrophages incubated with L. (L.) major lipophosphoglycan reduce in vitro proliferation of triple-negative breast cancer cells**

CORRÊA, G.M.B.; PROFETA, A.J.M.; NASCIMENTO, C.S.; DA SILVA, P.D.C.; SILVA, C.E.C.; MARTINS-FILHO, O.A.; DE OLIVEIRA, J.G.; PASCOAL-XAVIER, M.A.; BICALHO, K.A.; SOARES, R.P.P.; ALVES, É.A.R..
FIOCRUZ-MINAS, BELO HORIZONTE - MG - BRAZIL.

Conventional breast cancer treatments often show limited efficacy and cause significant adverse effects. In this challenging context, immunotherapeutic strategies targeting the tumor microenvironment have gained increasing attention, especially TLR2 agonists, which can reprogram macrophages from the immunosuppressive M2 phenotype to the pro-inflammatory, antitumoral M1 phenotype. Lipophosphoglycan (LPG) from *L. (L.) major*, a membrane-associated glycolipid, interacts with TLR2 and triggers strong inflammatory responses in macrophages, suggesting its potential as an immune modulator in cancer. This study evaluated whether LPG induces M2-to-M1 macrophage reprogramming in an in vitro breast cancer model and assessed its effects on tumor cell proliferation and viability. Human macrophages were conditioned with supernatants from MDA-MB-231 cells for 72 hours, followed by treatment with LPG (10 µg/mL) for 72 and 96 hours. Culture supernatants were collected to measure cytokine levels (flow cytometry), nitric oxide (NO), and hydrogen peroxide (H_2O_2) (colorimetric assays). Cell viability (MTT and Trypan blue assays) and macrophage phenotype (flow cytometry) were also assessed. Additionally, the effects of macrophage-derived supernatants and the direct effects of LPG on MDA-MB-231 cells were analyzed. LPG was non-toxic to macrophages, which retained a hybrid M1/M2 phenotype post-conditioning, with no significant changes after LPG exposure. Notably, supernatants from LPG-treated macrophages significantly reduced MDA-MB-231 proliferation, while LPG alone had no direct effect on tumor cells, suggesting an antitumor effect mediated by macrophages. Although cytokine, NO, and H_2O_2 analyses are ongoing, preliminary data indicate that LPG may promote macrophages' release of soluble factors capable of modulating tumor proliferation, supporting its potential as an immunotherapeutic agent in breast cancer.

Supported by: FAPEMIG (APQ-04360-23, APQ-04995-23); ReMPTO (RED-059/2023); CNPq/Fiocruz (Bolsa PIBITI); Rede de Plataformas Tecnológicas da Fiocruz
Keywords: Macrophages;Breast Cancer;Lipophosphoglycan.

TB – 006 - **Unraveling the role of m6A in antimony resistance in *Leishmania***

REIS, A.H.[1]; MARINO, A.G.[1]; GARCIA, M.A.D.N.[1]; FRESQUI, S.D.F.M.[1]; MENESES, A.V.I.[2]; MACHADO, F.C.[1]; PRADA, C.F.[2]; NETO, R.L.D.M.[3]; COELHO, A.C.[4]; VASCONCELOS, E.J.R.[5]; MORETTI, N.S.[1].
1. UNIFESP, SÃO PAULO - SP - BRAZIL; 2. UNIVERSITY OF MONTREAL, SAINT-HYANCINTHE - CANADA; 3. FIOCRUZ, BELO HORIZONTE - MG - BRAZIL; 4. UNICAMP, CAMPINAS - SP - BRAZIL; 5. UNIVERSITY OF LEEDS, LEEDS - UNITED KINGDOM.

Leishmania species are the etiological agents of leishmaniasis, a group of diseases that affect millions of people each year in about 100 countries. The treatment of leishmaniasis mainly relies on antimonials (Sb), miltefosine, and amphotericin B. Although treatments are available, the emergence of resistant parasites is common and often associated with changes in gene expression and genome architecture. Clinical isolates naturally resistant to Sb often show reduced expression of AQP1 and increased expression of genes related to oxidative stress response. In *Leishmania*, gene expression is primarily regulated post-transcriptionally, through mRNA stability/degradation and translation. Chemical RNA modifications have emerged as important post-transcriptional regulatory mechanisms, including N6-methyladenosine (m6A), which affects all aspects of RNA fate. m6A is added by METTL3/METTL14 and removed by FTO/ALKBH5. m6A has been found in *Plasmodium falciparum*, *Toxoplasma gondii* and *Trypanosoma brucei*, where it regulates parasite stage differentiation. Our group confirmed the presence of m6A and quantified its levels in five *Leishmania* species. In *L. mexicana* and *L. infantum*, m6A levels varied across life stages, with a notable increase in the amastigote form. To investigate the impact of m6A on drug resistance, we compared Sb-sensitive and Sb-resistant *L. infantum* strains and found reduced m6A levels in resistant parasites a phenotype reversed when parasites were cultured without drug pressure. Using direct RNA sequencing, we mapped m6A-modified transcripts in Sb-resistant and -sensitive *L. infantum* and identified over 4,000 methylated transcripts, including AQP1, ODC, TRYR, TRYP, and ABC transporters. These findings highlight m6A as a key post-transcriptional regulator in *Leishmania* and suggest its direct role in antimony resistance phenotype in *L. infantum*.

Supported by: FAPESP 2025/06325-6
Keywords: Leishmania;m6A;RNA modification.

TB – 007 - **Functional analysis of a gene potentially involved in miltefosine resistance in *Leishmania amazonensis***

DOS SANTOS, T.R.; AGOSTINO, V.D.S.; COELHO, A.C.. UNIVERSIDADE ESTADUAL DE CAMPINAS, CAMPINAS - SP - BRAZIL.

Cutaneous leishmaniasis is a neglected tropical disease that affect around 20,000 people per year in Brazil, which is mainly caused by *Leishmania braziliensis*, *L. guyanensis* and *L. amazonensis*. The later species is responsible to two main clinical forms of cutaneous leishmaniasis: localized cutaneous and diffuse cutaneous leishmaniasis. Treatment of disease is a public health challenge due to limited number of drugs available and the parasite's genetic plasticity, which may lead to drug resistance One of the drugs available for the treatment of leishmaniasis is miltefosine, whose mechanism of action and resistance is not completely understood, particularly in endemic species in Brazil. Recently, we sequenced the genome of a *L. amazonensis* resistant line to miltefosine and bioinformatic analysis identified mutations in genes possibly related to the resistance phenotype. Among these mutations, a heterozygous mutation was detected in the gene encoding lanosterol synthase (LS), an enzyme that plays a key role in the biosynthesis of ergosterol, the main sterol of the parasite’s plasma membrane. In order to functionally validate the role of this enzyme in miltefosine resistance, we propose to use reverse genetics techniques to evaluate the impact of this mutation in miltefosine
resistance phenotype in *L. amazonensis*. The LS gene has been amplified and cloned in an expression vector to investigate its role in miltefosine resistance. Additionally, using CRISPR/Cas9 technology, mutant knockout lines for LS gene have been also generated to evaluate whether LS is involved in resistance phenotype. Once transgenic lines were genetically validated, they were evaluated through of miltefosine susceptibility assays at promastigote and intracellular amastigote stages. These findings will contribute for a better understanding of the mechanism of action and resistance to miltefosine in Leishmania.

Keywords: *Leishmania amazonensis*, drug resistance, miltefosine.
Financial support: FAPESP and CNPq.
Supported by: FAPESP (2024/11197-4) e CNPq
Keywords: Leishmania amazonensis;drug resistance;miltefosine.

TB – 008 - ***In silico* and *in vitro* evaluation of indirubins as potential therapeutic agents against *Tritrichomonas foetus*.**

MACHADO, A.P.O.; COSTA, D.D.S.; GRAZUL, R.M.; JAEGER, L.H..
UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA - MG - BRAZIL.

Tritrichomonas foetus is a protozoan that infect animals, causing abortions and infertility in cattle, resulting in economic losses for livestock farming. There is no effective treatment, highlighting the need to identify new therapeutic alternatives for bovine trichomoniasis. This study aims to evaluate the *in vitro* and *in silico* biological activity of synthetic indirubins against *T. foetus* and to predict *in silico* physicochemical, pharmacokinetic, and toxicological parameters. *In vitro* activity of the indirubins (n=4) was evaluated against trophozoites at different concentrations (max. concentration 100 µM) and cell viability (inhibitory concentration of 50%, IC50) was determined after addition of Trypan blue and counting in a hemocytometer. *In silico* evaluation of parasitic, antiprotozoal, and trichomonacidal activity was performed using the Way2Drug platform (predicted activity >30%) and pharmacokinetic and physicochemical parameters were analyzed using the SwissADME platform. Toxicological parameters (hepatotoxicity, AMES toxicity, and skin sensitivity) were obtained using the pkCSM Pharmacokinetics platform. Metronidazole (MTZ) was used as the positive control. Two synthetic indirubins showed *in vitro* anti-*T. foetus* activity, with IC50 values of 33 and 29 µM. *In silico* prediction of biological activity did not show antiparasitic or trichomonacidal effects for the compounds. The compounds showed low TPSA values, indicating liposolubility (better absorption) and good bioavailability when administered orally (without violating Lipinski's or Veber's rules). Potential mutagenicity was observed for all indirubins and the positive control MTZ. No hepatotoxicity or skin sensitivity was identified. This study contributes to the development of therapeutic alternatives that may reduce economic losses in livestock.

Supported by: Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, APQ-N-000149/23)
Keywords: Tritrichomonas foetus;in vitro anti-Tritrichomonas foetus activity;in silico pharmacokinetics parameters.

TB – 009 - **NEW FRONTIERS IN ANTITRYPANOSOMAL DRUG DISCOVERY: POTENTIAL OF MICROBIAL METABOLITES FROM MARINE BACTERIA AND EXTREMOPHILES FROM PATAGONIA**

SILVA, V.A.[1]; LAGO, J.H.G.[2]; SANTOS, C.[3]; PIMENTA, D.C.[1]; TEMPONE, A.G.[1].
1. INSTITUTO BUTANTAN, SÃO PAULO - SP - BRAZIL; 2. UNIVERSIDADE FEDERAL DO ABC, SÃO PAULO - SP - BRAZIL; 3. UNIVERSIDAD LA FRONTERA, TEMUCO - CHILE.

Chagas disease, caused by the *Trypanosoma cruzi* parasite, affects approximately 8 million people worldwide and is an important public health problem. The current treatment is limited to a single toxic and ineffective drug in Brazil, highlighting the urgent need for new therapies. Microbial natural products have been widely used as scaffolds in drug discovery. Marine and extremophilic microorganisms, adapted to extreme environments, produce a vast chemodiversity, with promising biological activities. This study evaluated the antitrypanosomal potential of metabolites from extremophiles isolated from sediments of the Grey Glacier Lake and the hypersaline Bitter Lagoon (Chilean Patagonia), and marine bacteria from the São Paulo coast (Brazil). Bacteria were isolated using conventional methods and identified by MALDI-ToF/MS and 16S rRNA gene sequencing. The bacterial metabolites of 15 bacteria were extracted with organic solvents, and the anti-*T. cruzi* activity was evaluated against trypomastigotes for 24h incubation, using resazurin. The most promising extract was selected for bio-guided fractionation and chemical analysis, using RP-HPLC and ^{1}H-NMR and LC-MS/MS. All organic extracts killed 100% of the parasites, with IC_{50} values between 1 and 58 µg/mL. The most promising extract (PLASE1.1) was selected for bio-guided fractionation, revealing a mixture of low-molecular-weight compounds containing aromatic rings and fatty acids. The RP-HPLC peaks of the hexane fractionation resulted in one compound with anti-*T. cruzi* activity, with a molecular weight of 349 g/mol. The methanolic fraction resulted in 20 peaks, with 13 actives against the parasite. Marine and extremophilic microorganisms represent promising sources of novel antitrypanosomal compounds and could be used as future scaffolds for the design of new drugs for Chagas disease.

Supported by: FAPESP 2024/16243-4 e CAPES
Keywords: Trypanosoma cruzi;Therapy;Natural Products.

TB – 010 - **Iron Oxide Nanoparticles: Contrasting Effects in Cutaneous Leishmaniasis**

DA SILVA, P.D.C.[1]; LIMA, B.D.M.[2]; PROFETA, A.J.M.[1]; NASCIMENTO, C.S.[1]; LAGE, A.C.P.[1]; DE MELO, C.P.[3]; SILVA, C.E.C.[1]; ALVES, É.A.R.[1].
1. FIOCRUZ-MINAS, BELO HORIZONTE - MG - BRAZIL; 2. UFMG, BELO HORIZONTE - MG - BRAZIL; 3. UFPE, RECIFE - PE - BRAZIL.

Currently available treatments for cutaneous leishmaniasis (CL) are often associated with high toxicity and recurrent therapeutic failures, highlighting the urgent need for new strategies against this neglected disease. Iron oxide nanoparticles (NPs) have emerged as promising alternatives due to their antiparasitic effects, low toxicity, and good biocompatibility. This study evaluated the antiparasitic and immunomodulatory effects of maghemite nanoparticles encapsulated with polyaniline (Pani/γ-Fe_2O_3) in in vitro and in vivo CL models. In vitro, 5 × 10^6 *Leishmania (L.) amazonensis* promastigotes were exposed to Pani/γ-Fe_2O_3 NPs (60 µg/mL) for 24 h; viability was assessed by trypan blue exclusion. THP-1 macrophages infected with *L. (L.) amazonensis* were treated similarly, and infection index, intracellular parasite viability, nitric oxide, cytokines, and macrophage phenotypes were evaluated. NPs were cytotoxic to promastigotes and reduced parasite burden in macrophages without activating macrophage microbicidal functions, as cells remained M0 with low NO and IL-10 production. Given these positive in vitro results, infected BALB/c mice were treated intralesionally with 12.5 µg NPs every 4 days for 20 days. Lesion size and body weight were monitored, and blood, lesions, and spleens were analyzed. Treatment failed to reverse disease, instead increasing parasite burden, splenomegaly, lymphopenia, monocytosis, and extramedullary hematopoiesis, suggesting immunosuppressive or pro-inflammatory effects of the IONP formulation. Pani/γ-Fe_2O_3 NPs showed antiparasitic activity in vitro but worsened disease and altered immune responses in vivo, indicating the need for further optimization before therapeutic use.
Supported by: Fiocruz-Minas; Rede de Plataformas Tecnológicas da Fiocruz
Keywords: Leishmaniasis;Iron oxide;Nanoparticles.

TB – 011 - **Evaluation of a Novel *Crithidia fasciculata*-Based Vaccine Platform for the Control of Bovine Trypanosomosis**

ROSSINI, L.L.[1]; DÍAZ, G.[1]; GAMBA, J.C.[1]; MARCIPAR, I.[1]; ARIAS, D.[2]; BONTEMPI, I.[1].
1. LABORATORIO DE TECNOLOGÍA INMUNOLÓGICA. FACULTAD DE BIOQUÍMICA Y CIENCIAS BIOLÓGICAS. UNIVERSIDAD NACIONAL DEL LITORAL, SANTA FE - ARGENTINA; 2. LABORATORIO DE ENZIMOLOGÍA MOLECULAR. INSTITUTO DE AGROBIOTECNOLOGÍA DEL LITORAL. CONICET, SANTA FE - ARGENTINA.

Bovine trypanosomosis**,** caused by the hemoparasite *Trypanosoma vivax*, remains a significant challenge to livestock production in Africa and South America. A prophylactic vaccine capable of mitigating parasite pathogenicity could markedly improve animal health and production in endemic regions. The study evaluated an innovative veterinary vaccine platform based on *Crithidia fasciculata*, a non-pathogenic kinetoplastid. This organism has been adapted as a recombinant system to express and deliver *T. vivax* antigens. Two immunogenic targets from *T. vivax* were selected and tested independently: Tv45, an invariant surface glycoprotein (ISG) family, and Tv1, an enzyme implicated in pathogenicity and considered a key virulence factor. For each antigen, the corresponding sequence was cloned into the pNUSHcH vector and used to transform *C. fasciculata* by electroporation. Recombinant strains, maintained under hygromycin selection, were evaluated separately in BALB/c mice. Both candidates were found to be safe, inducing antigen-specific antibodies (p<0.01 for 1-CF and p<0.02 for 45-CF) and significantly stronger delayed-type hypersensitivity responses (p<0.001 for 1-CF and p<0.0009 for 45-CF) compared to controls. Following challenge with *T. vivax* strain Y486, survival reached 75% for Tv45 and 50% for Tv1, while all control mice succumbed by day 30 post-infection. Both vaccines significantly reduced early-stage parasitemia and mitigated clinical severity. These results support the potential development of a vaccine against *T. vivax* and position *C. fasciculata* as a promising platform for veterinary immunization.
Keywords: Trypanosoma vivax;Recombinant Vaccine;Invariant Surface Glycoprotein.

TB – 012 - **Targeting Ergosterol and Cholesterol Pathways: In vitro Activity of Ravuconazole with Atorvastatin Against Trypanosoma cruzi**

PEREIRA, G.M.[1]; FERNANDES, V.Â.[1]; VERDAGUER, I.B.[2]; CALDAS, I.S.[1]; DINIZ, L.D.F.[1].
1. UNIVERSIDADE FEDERAL DE ALFENAS, ALFENAS - MG - BRAZIL; 2. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRAZIL.

The scarcity of effective new anti-*Trypanosoma cruzi* agents highlights the need to evaluate clinically approved drugs as potential therapies for Chagas disease. While ergosterol biosynthesis inhibitors have proved ineffective in eliminating *T. cruzi* infection as monotherapies, evidence suggests that adipose tissue serves as a parasite reservoir and that *T. cruzi*'s ability to scavenge host lipids is critical for intracellular survival. Here, we investigated the *in vitro* anti-*T. cruzi* activity of the ergosterol biosynthesis inhibitor ravuconazole combined with atorvastatin, a clinically used hypocholesterolemic agent. HepG2 cells were used to assess cytotoxicity via resazurin reduction assay. Anti-*T. cruzi* (Y strain) activity was tested against tissue-derived trypomastigotes and amastigotes using HepG2 cells as hosts. Trypomastigote inhibition was determined by optical microscopy after 24 h incubation; amastigote activity was assessed in a qualitative 96-well plate assay after 72 h drug exposure followed by washout to detect suppression or reactivation of parasitism. We found that ravuconazole exhibited no cytotoxicity up to 1 µM, whereas atorvastatin was toxic above 25 µM. When combined, ravuconazole did not influence atorvastatin's toxicity. Regarding anti-*T. cruzi* activity, both drugs were inactive against trypomastigote forms, either as monotherapies or in combination. In infected cell assays, atorvastatin at 12.5 µM slightly reduced the number of released trypomastigotes, but its combination with ravuconazole did not potentiate this effect. Our findings suggest that atorvastatin does not enhance the anti-*T. cruzi in vitro* activity of ravuconazole. Studies evaluating the impact of host cholesterol levels on ravuconazole effect are ongoing.
Supported by: CAPES, FAPEMIG, UNIFAL
Keywords: Chagas disease;drug combination;lipidic metabolism.

TB – 013 - **The Role of RBP23 in Gene Expression Regulation: Potential Protein Partners and mRNAs Targets in *Trypanosoma brucei***

FALCÃO, S.S.F.; DE ASSIS, L.A.; MOURA, D.M.N.; NETO, O.P.D.M..
INSTITUTO AGGEU MAGALHÃES/FUNDAÇÃO OSWALDO CRUZ, RECIFE - PE - BRAZIL.

Trypanosoma brucei is a well-known model organism for the investigation of gene regulation in trypanosomatids, mainly controlled post-transcriptionally. Translation initiation is likely a major regulatory step, generally involving the eukaryotic initiation factors (eIFs). One major target for translation regulation is the eIF4F complex, formed by joining eIF4E and eIF4G subunits, which promotes mRNA recognition and ribosome recruitment for translation. Multiple homologues for the eIF4F subunits are found in trypanosomatids forming different eIF4F-like complexes. Different complexes demonstrate a preferential association for specific sets of mRNAs, assisted by various RNA-binding proteins (RBPs). The *Leishmania* RBP23 was identified in mass-spectrometry results as a potential participant in translation control. This work aimed to characterize RBP23 in *T. brucei* through identification of partner proteins and mRNA targets, through immunoprecipitation (IP) followed by mass-spectrometry and RNA-seq, respectively. Proteomic analysis confirmed the strong association between RBP23 and a polyadenylate-binding protein homologue, PABP1, echoing the findings in *L. infantum* but now confirmed in *T. brucei*. RBP23 also co-precipitates with the EIF4E4/EIF4G3 complex and proteins involved in translation regulation, such as RNA helicases (DHH1), ZC3H41, and ALBA-domain proteins. The transcriptomic analysis identified over 130 mRNAs with RBP23 encoding ribosomal proteins. This corroborates the hypothesis that RBP23 preferentially recruits ribosomal mRNAs for translation by binding to these RNAs and mediating their interaction with the EIF4E4/EIF4G3 complex. Motif discovery in the 3'UTRs of the top 30 ribosomal mRNAs identified a highly significant U-rich motif. This recapitulates the U-rich elements previously described in similar *L. infantum* transcripts, reinforcing the possibility that RBP23 mediates selective translation through conserved sequence elements in the mRNAs' 3'UTRs.
Supported by: CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico. Processo nº 141438/2023-1
Keywords: Trypanosomatids;Gene expression regulation;RNA-Binding Proteins.

TB – 014 - ***In vivo* anti-*Leishmania amazonensis* effects of topical and systemic treatment in mice with ulcerated lesions using the metal complex compound A3310 combined with protein hydrogel**

REZENDE, L.M.[1]; MOREIRA, F.F.[1]; FERNANDES, C.[2]; JÚNIOR, A.H.[2]; PEREIRA, M.F.[1]; DE CARVALHO, E.C.Q.[1]; DAMATTA, R.A.[1]; SEABRA, S.H.[1].

1. UENF-UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE DARCY RIBEIRO, CAMPOS DOS GOYTACAZES - RJ - BRAZIL; 2. UFSC- UNIVERSIDADE FEDERAL DE SANTA CATARINA, FLORIANÓPOLIS - SC - BRAZIL.

Cutaneous leishmaniasis is a neglected disease caused mainly by *Leishmania amazonensis*, the primary etiological agent in Brazil. Conventional treatment involves pentavalent antimonials and amphotericin B (AmB), both face limitations due to toxicity and parasite resistance, reinforcing the need for new therapies. The metal complex, formed by a metal core coordinated to organic ligands, has demonstrated *in vitro* efficacy against *L. amazonensis* in axenic studies, emerging as a promising therapeutic alternative. This study evaluated, *in vivo*, the efficacy of the cobalt-based metal complex A3310 in BALB/c mice infected in the auricular pavilion with amastigotes of *L. amazonensis* (Josefa strain), resulting in ulcerated lesions. Mice were treated for 40 days, combining topical and systemic approaches, divided into five groups: Control; Hydrogel + A3310 (100 or 200 µg/kg); Hydrogel + Amphotericin B (200 µg/kg); and Hydrogel alone. Wound healing, and lesion size; and post-treatment parasite load were assessed using calipers and histological analysis, respectively. A3310 treatment progressively reduced lesions, with a more pronounced effect at 100 µg/kg compared to AmB. Additionally, tissue formation over the lesions showed tissue regeneration compared to control and AmB-treated groups. Histopathological analyses revealed reduced parasite load and evident tissue recovery in A3310-treated groups, characterized by mild inflammatory infiltrate and restoration of dermal features. In contrast, the control, hydrogel alone, and AmB groups exhibited tissue disorganization, intense inflammatory infiltrate, and high parasite load. These findings highlight the therapeutic potential of A3310, administered systemically and topically using hydrogel as a vehicle, for the treatment of cutaneous leishmaniasis, with greater efficacy than AmB, combining antiparasitic effects and promoting tissue regeneration.

Supported by: CAPES, CNPq e FAPERJ

Keywords: Cutaneous leishmaniasis;Leishmania amazonensis;Metal complex.

TB – 015 - **Effects of Chronic Toxoplasma gondii infection on a Cuprizone-Induced Multiple Sclerosis Model**

DA CRUZ, S.A.A.[1]; DE FREITAS, V.P.[1]; PEREIRA, A.C.D.P.A.[1]; MELO,, E.D.D.N.[2]; GOMES, L.D.E.S.[1]; DO NASCIMENTO, G.C.[1]; CAMPOS, M.R.D.[1]; PINTO, V.B.[1]; ADESSE, D.[3]; SEABRA, S.H.[1]; DAMATTA, R.A.[1]; ARAÚJO, S.E.S.[1].

1. UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE DARCY RIBEIRO, CAMPOS DOS GOYTACAZES - RJ - BRAZIL; 2. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO - MACAÉ, CAMPOS DOS GOYTACAZES - RJ - BRAZIL; 3. FIOCRUZ - RJ, CAMPOS DOS GOYTACAZES - RJ - BRAZIL.

Multiple Sclerosis (MS) is an autoimmune, demyelinating, and inflammatory disease of the central nervous system that causes cognitive, sensory, and motor symptoms. It is estimated to affect approximately 3 million people worldwide, with higher prevalence in developed countries. In contrast, Toxoplasma gondii infections are more common in less developed countries. Recent studies have reported a negative correlation between chronic T. gondii infection and MS, suggesting a possible protective effect of the infection. In this study, we evaluated the impact of chronic T. gondii (ME49 strain) infection in C57BL/6 mice subjected to the cuprizone-induced demyelination model, which mimics MS. After 12 weeks of infection, animals were treated with cuprizone for 5 weeks. Four experimental groups were used: control (CTL); infected control (CTL+Tg); cuprizone-treated (CUP); and cuprizone-treated and infected (CUP+Tg). Behavioral assessments included memory (novel object recognition – NOR), anxiety (open field), motor coordination (Rotarod), and tactile sensitivity (von Frey) tests. Subsequently, the cerebral cortex was collected for Western blotting and proteomic analyses. In the NOR test, CUP showed a trend toward memory impairment, which was significantly reversed in CUP+Tg. In the von Frey test, CTL+Tg exhibited greater sensitivity than CTL, CUP showed reduced sensitivity, and CUP+Tg recovered to CTL levels. We observed reduced CNPase (a myelin protein) levels in CUP vs. CTL, with no difference between CUP and CUP+Tg. These findings suggest that chronic T. gondii infection may exert a protective effect against MS pathophysiology in the cuprizone model, but apparently remyelination is not part of the involved mechanism.

Supported by:

Keywords: Multiple Sclerosis;Toxoplasma gondii;Cuprizone.

TB – 016 - **Towards vaccine implementation for bovine trypanosomosis: Tv1 immunization achieves long-term protection**

DÍAZ, G.[1]; ROSSINI, L.L.[1]; ARIAS, D.[2]; MARCIPAR, I.[1]; BONTEMPI, I.[1].
1. LABORATORIO DE TECNOLOGÍA INMUNOLÓGICA - FACULTAD DE BIOQUÍMICA Y CIENCIAS BIOLÓGICAS - UNIVERSIDAD NACIONAL DEL LITORAL, SANTA FE - ARGENTINA; 2. LABORATORIO DE ENZIMOLOGÍA MOLECULAR - INSTITUTO DE AGROBIOTECNOLOGÍA DEL LITORAL - CONICET, SANTA FE - ARGENTINA.

Bovine trypanosomosis, caused by *Trypanosoma vivax* (*T. vivax*), results in substantial economic losses across Africa and South America. Infection is characterized by intermittent parasitemia and severe anemia, often resulting in death. The development of vaccines that target the parasite's pathogenic mechanisms represents a critical step toward effective disease control. In this study, we evaluated several invariant surface glycoproteins and derived virulence factors of *T. vivax* previously identified as promising vaccine candidates, in a murine model. Preliminary assessments of parasitemia, hematocrit and body weight loss identified Tv1 as the most promising candidate. Following optimization of formulation parameters, including antigen dose, antigen load and adjuvant composition, immunization with Tv1 formulated with adjuvant 3 (Tv1-Adj3) conferred long-lasting protection against a lethal *T. vivax* challenge. The protection was evidenced by a substantial decrease in mortality and an enhancement in clinical outcomes. Furthermore, Tv1-Adj3 elicited robust antigen-specific antibody responses, with an IgG2a-inclined IgG2a/IgG1 ratio. Passive transfer of these antibodies protected naïve mice from infection. Moreover, immunization with Tv1-Adj3 promoted the production of IFN-γ, TNF-α and IL-10 and prevented the reduction of splenic B cell populations, typically observed in *T. vivax*-infected animals. These findings further support Tv1 as a promising vaccine candidate with the potential to mitigate the clinical manifestations of *T. vivax* infection. This work establishes a basis for the development of a subunit vaccine to control bovine trypanosomosis, reducing dependence on trypanocidal drugs and mitigating associated economic losses. Subsequent studies will focus on the effectiveness, safety and scalability of the vaccine in bovine hosts under field conditions.

Keywords: Trypanosoma vivax;Animal trypanosomiasis;subunit vaccine.

TB – 017 - **Preclinical Evaluation of Indomethacin as an Anti-*Toxoplasma* Agent**

DIAS, I.D.O.; MOURA, G.C.; REIMAO, J.Q..
FACULDADE DE MEDICINA DE JUNDIAÍ, JUNDIAÍ - SP - BRAZIL.

Toxoplasmosis is a worldwide zoonosis caused by the obligate intracellular protozoan *Toxoplasma gondii*, affecting approximately one-third of the human population. Although most infections remain asymptomatic in immunocompetent individuals, the disease can cause severe and potentially fatal manifestations in neonates and immunocompromised patients. Conventional treatment presents important limitations, including severe adverse effects and poor efficacy against chronic stages of infection. Drug repurposing emerges as a promising strategy, enabling the exploration of compounds already approved for other indications, thereby reducing both development time and cost for new antiparasitic agents. This study aimed to evaluate the *in vitro* and *in vivo* anti-*T. gondii* activity of indomethacin in an acute toxoplasmosis model using the RH strain. The drug showed a Half Effective Concentration (EC_{50}) of 1.2 µM against intracellular tachyzoites and of 23.5 µM against human foreskin fibroblasts. For the in vivo efficacy assessment, mice were intraperitoneally infected with 2.5×10^5 tachyzoites. Treatment began 24 hours post-infection, with the experimental group receiving indomethacin (10 mg/kg/day) orally for 10 consecutive days, while the control group received only the vehicle (n = 5 per group). Animals were monitored daily for clinical signs and mortality. Indomethacin treatment resulted in an average survival extension of three days compared with the control group. Although the effect was insufficient for complete cure or control of infection in this model, the findings suggest potential *in vivo* activity of indomethacin against *T. gondii*. A follow-up experiment is underway, evaluating a twice-daily dosing regimen to increase systemic exposure and potentially enhance therapeutic efficacy. These results highlight the potential of drug repurposing as a strategy for discovering new drug candidates for toxoplasmosis and support further preclinical investigation of indomethacin.

Supported by: FAPESP (process numbers 2018/18954-4 and 2024/22547-6)
Keywords: Toxoplasma gondii;drug repurposing;preclinical studies.

TB – 018 - **Structure-Guided Discovery of Adenylosuccinate Lyase Inhibitors for Visceral Leishmaniasis**

ALVES, B.C.R.; LINO, M.E.S.D.; THIEMANN, O.H..
UNIVERSIDADE DE SÃO PAULO, SÃO CARLOS - SP - BRAZIL.

Visceral leishmaniasis (VL), caused by the protozoan *Leishmania donovani*, is a severe neglected tropical disease, primarily affecting vulnerable populations. Current therapeutic options present high toxicity, elevated costs, the need for hospitalization, and increasing parasite resistance, highlighting the urgent need for the development of new selective drugs. The enzyme adenylosuccinate lyase (ASL) plays a crucial role in nucleotide biosynthesis and is essential for the survival of *Leishmania* spp., since kinetoplastids, unlike humans (*Hs*ASL), lack the *de novo* purine synthesis pathway. Thus, ASL from *Leishmania major* (*Lm*ASL) and *L. donovani* (*Ld*ASL) represents promising targets, as their inhibition can specifically disrupt the parasite's life cycle, with potential applications against trypanosomiasis as well.
In the present study, molecular docking assays were performed using the GOLD software, screening 400 compounds from different classes (antifungals, antivirals, and antibacterials) against *Lm*ASL and its homologs (*Hs*ASL and *Tb*ASL). To further address this results the best-performing compounds will be subjected to co-crystallization trials and enzymatic activity assays.
The experimental methodology involved recombinant expression of *Ld*ASL in *Escherichia coli*, followed by biophysical characterization (DLS, SEC-MALS, CD). Biochemical evaluations (kinetic and inhibition assays) for functional validation are currently in progress. At this stage, the protein has already been successfully cloned, expressed, and purified. The next steps include further biochemical and biophysical characterization, *in vitro* screening of novel compounds and to determine antiparasitic activity, cytotoxicity, and selectivity, as well as co-crystallization and X-ray diffraction experiments to elucidate protein–inhibitor interactions.
By integrating biophysical and structural biology, biochemistry, medicinal chemistry, and molecular simulation approaches, this project aims to contribute to the discovery of innovative drug candidates against VL and to strengthen the scientific training of the researcher involved.
Supported by: Não
Keywords: Visceral Leishmaniasis;Adenylosuccinate Lyase;Drug Discovery.

TB – 019 - **Integration of vaccination schemes based on TS and TcTASV antigen families developed by two Argentine groups: Potential improvement in protection against challenge with two *Trypanosoma cruzi* strains**

DÍAZ, G.[1]; CACIK, P.[1]; MOLINARI, P.[2]; MASIP, Y.E.[3]; COSENZA, M.[3]; CHA, U.[1]; VEINTICINQUE, L.[1]; PEREZ, A.R.[4]; MARCIPAR, I.[1]; TEKIEL, V.[3].
1. LABORATORIO DE TECNOLOGÍA INMUNOLÓGICA - FACULTAD DE BIOQUÍMICA Y CIENCIAS BIOLÓGICAS - UNIVERSIDAD NACIONAL DEL LITORAL, SANTA FE - ARGENTINA; 2. INSTITUTO DE AGROBIOTECNOLOGÍA Y BIOLOGÍA MOLECULAR (IABIMO) - INSTITUTO NACIONAL DE TECNOLOGÍA AGROPECUARIA (INTA) - CONSEJO NACIONAL DE INVESTIGACIONES CIENTÍFICAS Y TÉCNICAS (CONICET), BUENOS AIRES - ARGENTINA; 3. INSTITUTO DE INVESTIGACIONES BIOTECNOLÓGICAS (IIB) - EBYN - UNSAM - CONICET, SAN MARTÍN - ARGENTINA; 4. INSTITUTO DE INMUNOLOGÍA CLÍNICA Y EXPERIMENTAL DE ROSARIO (IDICER) - CONICET, ROSARIO - ARGENTINA.

Two Argentine research groups independently developed *Trypanosoma cruzi* vaccine formulations that demonstrated strong protection in murine models. The UNL group designed a recombinant Group 1 trans-sialidase (rTS) vaccine with an ISCOMATRIX-like adjuvant (ISPA), administered in three homologous doses, achieving over 80% survival after lethal challenge with the Tulahuen strain compared to 0% in controls. The UNSAM group developed a heterologous protocol consisting of a priming dose with recombinant rTcTASV-C plus AlOH, followed by a boost with rTcTASV-C combined with recombinant baculoviruses expressing TcTASV-A in the capsid (BV::A-CAP), achieving over 90% survival against the highly virulent RA strain, compared to 0% in controls. This study evaluated two combined immunization schemes alongside the individual protocols, with experiments conducted in parallel in both laboratories using the Tulahuen and RA infection models. Cross-testing confirmed the robustness of each individual scheme. G4 (rTcTASV-C+rTS+AlOH → rTcTASV-C+rTS+BV::A-CAP) achieved the highest protection, with 100% survival versus 20% in controls ($p<0.01$; Tulahuen). Similar results were observed with the RA strain, where G3 (rTcTASV-C+rTS+ISPA → same → rTcTASV-C+rTS+BV::A-CAP) also reached 100% survival, and G1 (rTS+ISPA ×3) achieved 80% (vs 0% in controls). Also, mixed schemes generated the highest antibody titers against rTcTASV-C and TS and incremented vaccine antigen specific T cells. These findings demonstrate that the TS+ISPA and TASV+BV individual protocols are robust, that their integration effectively reduces parasitemia and achieves complete protection, and that comparable results across infection models highlight the strategy's consistency. The observed synergy between TS and TcTASV antigens, together with the collaborative work of both Argentine laboratories, underscores the potential of integrated vaccine strategies as a step toward an effective vaccine against Chagas disease.
Keywords: Vaccine;Chagas Disease;mixed schemes.

TB – 020 -**Standardization of a LAMP-PCR Assay with Homemade Bst Enzyme for *Leishmania amazonensis* Detection**

DE CARVALHO, R.L.; GUERRA, G.T.; DE GODOY, S.A.M.; QUEIROZ, B.O.; VENTURA, B.M.L.; MARQUEZINI, L.M.B.; DE OLIVEIRA, L.L.; VASCONCELLOS, C.M.M.; FIETTO, J.L.R.; VASCONCELLOS, R.D.S..
UNIVERSIDADE FEDERAL DE VIÇOSA, VIÇOSA - MG - BRAZIL.

Leishmaniases are anthropozoonotic diseases affecting over 1 million people annually, transmitted by phlebotomine sand flies infected with more than 20 *Leishmania* species. In Brazil, the recent discontinuation of the Montenegro skin test reagent created a diagnostic gap, particularly for the tegumentary form, affecting smaller municipalities without access to advanced diagnostic methods and requiring patient referrals to reference centers. This problem is aggravated by limitations of current tests, which may present cross-reactivity with other parasites and often fail to identify the infecting species. This study aimed to validate the use of *L. amazonensis* primers in a LAMP-PCR assay with a laboratory-produced ("homemade") Bst enzyme, as well as to standardize the reaction, evaluate specificity, and determine the detection limit. Genomic DNA from several *Leishmania* species, *T. cruzi* strains, and *T. rangeli* was extracted and quantified. Reactions were optimized by comparing enzyme sources (commercial vs. homemade), incubation temperatures (60–68 °C), $MgSO_4$ concentrations (5–15 mM), and reduced primer concentrations. Detection limits for *L. amazonensis* PH8 DNA were determined, along with limiting dilutions of the enzyme. Specificity tests included all extracted parasite DNAs, and colorimetric detection with malachite green was applied. Amplification performance was similar between commercial and homemade enzymes. Optimal conditions were 64–65 °C and 8–12 mM $MgSO_4$. The homemade enzyme retained activity after tenfold dilution. The assay detected as little as 100 fg of *L. amazonensis* DNA. Primers showed 100% specificity. Both colorimetric and gel-based detection allowed rapid interpretation, highlighting the primers' high sensitivity and specificity and their potential for species-specific diagnostic kit development.
Supported by: Fapemig, CNPq, CAPES
Keywords: Lamp-PCR;Leishmania amazonensis;Molecular diagnosis.

TB – 021 - **Nucleosome Architecture and Chromatin Organization in *Trypanosoma cruzi***

CASTRO, B.S.[1]; ISSA, M.[2]; DA CUNHA, J.P.C.[3]; THIEMANN, O.H.[1].
1. IFSC - USP, SÃO CARLOS - SP - BRAZIL; 2. CSSB, HAMBURGO - GERMANY; 3. BUTANTAN, SÃO PAULO - SP - BRAZIL.

Chagas disease, caused by ***Trypanosoma cruzi***, affects millions of people worldwide, with no available vaccines or adequate treatments. The parasite's infectivity and the processes that enable its survival involve gene expression regulation, especially at a post-transcriptional level. Recent evidence suggests that epigenetics plays a crucial role in its pathogenicity, inciting the study of chromatin structure and dynamics. The most basic level of chromatin packaging is the nucleosome, composed of DNA interacting around an octameric protein complex that contains a pair of each histone - H2A, H2B, H3, and H4. The oligomeric structure of the octamer from homologue microorganisms is composed of two dimers of H2A/H2B and one tetramer of H3/H4. This study aims at the biochemical and biophysical characterization of ***Trypanosoma cruzi*** histones, in addition to the atomic structure of the histone octamer and the nucleosome. Conditions of the recombinant expression of histone coding genes of ***T. cruzi*** in ***Escherichia coli*** T7 Express strain were determined. The four histone protein purification protocols included refolding techniques, affinity chromatography, and size-exclusion chromatography. The ***Tc***H2A/***Tc***H2B and ***Tc***H3/***Tc***H4 oligomers were obtained as heterodimers, diverging from homologue histones in which H3 and H4 assemble as a tetramer. The characterization of the histones and the nucleosome assembly are currently under development. Moreover, crystallization screening and SEC-SAXS assays were performed in order to investigate the structural organization of these oligomers, aiming to explain the differences obtained. The current results support the need to continue the research to properly obtain the structure of the ***T. cruzi*** octamer through Cryo-Electron Microscopy (Cryo-EM), as well as the kinetic and thermodynamic properties of nucleosome assembly.
Supported by: CNPq # 130068/2025-0
Keywords: Trypanosoma cruzi;Nucleosome;Cryo-EM.

TB – 022 - **LncRNAs in hematopoiesis and their potential role in *Leishmania infantum* chronic infection**

BAHIA, I.A.F.; DA FONSECA, D.L.M.; TEIXEIRA, C.A.; ZANATTA, J.M.; LIMA, C.N.C.; ELEUTERIO, B.P.; MUXEL, S.M..
UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRAZIL.

Introduction: Hematopoiesis is regulated by transcription factors, epigenetic and post-transcriptional control. lncRNAs help balance self-renewal and differentiation of hematopoietic stem/progenitor cells (HSPCs). Beyond physiology, the bone marrow is a key niche in chronic *Leishmania infantum* infection, the parasite persistence reshapes hematopoietic output, and this immune sanctuary has poor drug penetration, favoring recurrence. Despite advances in leishmaniasis, lncRNA roles remain underexplored.
Methodology: We screened 1,405 GEO datasets using Mus musculus, wild-type, bulk RNA-seq, and bone marrow filters, resulting in 39 studies (171 samples) of HSPC and marrow populations. Data underwent QC, pseudoalignment with Salmon, batch correction (ComBat-seq), and differential expression with DESeq2. Consistency was validated by PCA, clustering, and enrichment analyses.
Results and Discussion: Analyses showed clear segregation of progenitors and robust mRNA/lncRNA patterns. Enrichment confirmed canonical hematopoietic pathways; WGCNA resolved gene–lncRNA modules. Key transcripts included Gata2, Kit, Mpl, Notch1 (lineage commitment) and Fn1, Tgfb1 (niche remodeling). Among lncRNAs, Meg3, Mir99ahg, Lincred1, Hoxb3os, and Spehd (antisense of Gata2) were highlighted. We interpret these in light of genomic position and existing gene annotations. This is the first step of a roadmap integrating (i) basal hematopoiesis, (ii) monocyte-to-macrophage differentiation, and (iii) infection-induced remodeling in *L. infantum* models.
Conclusion: lncRNAs regulate hematopoietic networks and likely contribute to *L. infantum*-driven remodeling. Bridging basal hematopoiesis, lineage differentiation, and infection-induced change, our approach highlights candidate lncRNAs for functional testing with implications for host–parasite interaction and therapy, given their potential to modulate hematopoiesis.
Supported by: CAPES - 88887.992612/2024-00
Keywords: hematopoiesis;long-no-coding RNAs;leishmaniasis.