TB-08 - **The role of CD4⁺ T cell on the efficacy of intranasal LaAg vaccine**

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Leishmaniasis are neglected tropical disease caused by protozoan parasites of *Leishmania* genus. In Brazil, *L. amazonensis* is the main causative agents of cutaneous leishmaniasis. To mitigate the problems, the development of a vaccine against leishmaniasis is significant and urgent. But, at the moment, there isn't an approved vaccine to humans use. Previous studies with *L. amazonensis* antigens (LaAg) by intranasal route induced partial protection. Therefore, our aim is to characterize the mechanisms by which the LaAg vaccine acts in the immune system of mice, mainly the TCD4⁺ lymphocytes. For the accomplishment of the experiment C57BL/6 WT, $CD4^{-/-}$, $MHCII^{-/-}$ and Interferon-gamma$^{-/-}$ (IFN-$y^{-/-}$) mice were immunized twice with LaAg (0.5 µg/µL) intranasally with an interval of one week between them, as the control group, we used two doses of PBS. After one week of the second dose, the challenge was carried out by infecting the footpad of the mice with $2x10^5$ *L. amazonensis*, following the progression of the lesion measuring of the infected paw. At the end of the experiment, the parasite burden of the footpad and lymph nodes was quantified by LDA. Our results showed that vaccinated WT mice had a reduction in the lesion when compared to PBS. However, the vaccinated $CD4^{-/-}$ group mice didn't protect against the lesion or decrease of the parasite load, showing no statistical difference between the vaccinated and PBS groups mice. Similar results were obtained using $MHCII^{-/-}$ that demonstrated the importance of TCD4⁺ lymphocytes. We also vaccinated IFN-$y^{-/-}$, and no protection was observed in the lesion and parasite burden, suggesting the participation of IFN-y producing CD4+ T cells. Experiments using $RAG^{-/-}$ reconstituted with CD4⁺ IFN-$y^{+/+}$ or CD4⁺ IFN-$y^{-/-}$ T cells are necessary to confirm the role of this cell. We concluded that the efficacy of the LaAg vaccine is associated with the presence of TCD4⁺ lymphocytes which are essential for the control of disease in vaccinated mice. **Supported by:** CNPq N º 06/2019
Keywords: Leishmaniasis ;Vaccine ;CD4 T cell.

TB-09 - ***IN VITRO* AND *IN VIVO* CHARACTERIZATION OF A *Leishmania amazonenis* CLINICAL ISOLATE RESISTANT TO AMPHOTERICIN B**

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Leishmania amazonensis is the etiological agent of cutaneous and diffuse cutaneous leishmaniasis and it is considered one of the most relevant species in terms of incidence in Brazil. Treatment options for cutaneous leishmaniasis are restricted to pentavalent antimony, amphotericin B, pentamidine and miltefosine. The latter one is the only oral drug, approved for use in Brazil. Treatment failure has been reported and may be associated with several factors such as the immune response of the infected patient, co-infection with other pathogens, as the HIV virus, and factors directly related to the parasite, which drug resistance is the main factor. Here, we characterized a *L. amazonensis* clinical isolate from Northeast region of Brazil, whose patient was refractory to the treatment to two of the main drugs used against leishmaniasis in Brazil: pentavalent antimony and liposomal amphotericin B. *In vitro* drug susceptibility assays confirmed this isolate as resistant to both drugs at intracellular amastigote stage. These findings were compared with a susceptible strain to both drugs, confirming the treatment failure. The *in vivo* effectiveness of amphotericin B in BALB/c mice infected with this clinical isolate showed that animals were completely refractory to the highest dose of the drug. On the other hand, the *in vivo* effectiveness of miltefosine, paromomycin or a combination of both drugs showed that animals infected with this isolate responded to all treatment schemes, confirming the *in vivo* amphotericin B resistance phenotype for this clinical isolate. The molecular basis of amphotericin B and antimony resistance phenotype in this clinical isolate is under investigation. This study will provide data on the limitations and potential use of miltefosine, paromomycin and their combination as an alternative for the treatment against multiresistant isolates of *Leishmania*.
Supported by:FAPESP(2016/21171-6); CNPq (405235/2021-6); FAPESP (2022/06176-2)
Keywords: Leishmania amazonensis;drug resistance;amphotericin B.

TB-10 - **Cullin-RING ligase (CRL1) components assembles into a E3 ubiquitin-ligases complex in *Leishmania infantum***

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Cullin-RING ligases (CRL) are the largest and most studied class of E3 ligases in mammals being responsible for regulation of cell cycle and proliferation. CLR1 are a multiprotein complex composed by SKP1, Cullin 1, RBX1 and a F-box protein which interact with SKP1 through F-box domain and recruits the substrates. E3 ligases play a key role in ubiquitination process, recognizing and transferring ubiquitin to the substrate that might be directed to proteasome for degradation or processed by deubiquitinating enzymes. The Ubiquitin proteasome system (UPS) are the main regulator of intracellular proteolysis in eukaryotes. In parasitic protozoan, this process is essential for the alternation of hosts in their life cycles and consequently for success of parasitism. Little is known about *Leishmania spp.* UPS and no description about CRL in *L. infantum*, has been found. We showed through *in silico* analysis that predicted *L. infantum* genes SKP1-like protein, cullin-like protein-like and ring-box protein 1 have their interaction motifs conserved related to their orthologs in *Homo sapiens.* Co-immunoprecipitation assays demonstrated that these proteins are able to assemble in a CRL complex in this parasite. In addition, mass spectrometry of SKP1 and Cullin1 interactome of *L. infantum* protein extracts revealed protein partners of these baits related to different intracellular processes, such as nucleic acid binding and UPS components including proteasome subunit, an ubiquitin-like protein and six F-box proteins. Moreover, we generate by CRISPR/Cas9 a *L. infantum* strain expressing both 3xmyc-mCherry-Cullin1 and HA-SKP1 and demonstrated that these proteins interacted in promastigotes thorough co-immunoprecipitation. Thus, a new class of E3 ubiquitin-ligases has been described in *L. infantum* with functions related to different parasite processes. **Supported by:**FAPESP 2021/10971-0
Keywords: Cullin-RING ligase;Ubiquitin;Leishmania infantum.

TB-11 - **Characterization of miltefosine transporter gene of *Leishmania (Viannia) braziliensis:* genomic organization and functional analysis using reverse genetic tools**

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In Brazil, *L. (V.) braziliensis* is the most prevalent species, responsible for cutaneous leishmaniasis (CL). Miltefosine (MF) was approved in Brazil a few years ago to treat CL and have been presenting some benefits like oral administration and less side effects when compared with the currently used drugs. MF accumulation is mediated by a complex of proteins located in the plasma membrane that include miltefosine transporter (MT) and Ros3. Several studies have associated the role of these proteins in MF susceptibility and resistance in some species of *Leishmania*, but little is known about the role of MT in *L. (V.) braziliensis*. The genome resequencing of *L. (V.) braziliensis* (M2903 strain), using a combination of Illumina and Nanopore technologies, revealed that the *MT* gene has three copies in chromosome 13 of this triploid organism. Functional characterization of MT in this species was performed generating of null mutants and by overexpression of *MT* gene in a resistant line of *L. (L.) amazonensis,* whose *MT* gene is not functional, due to a mutation in its coding sequence. Null mutants for the *L. (V.) braziliensis MT* gene were highly resistant to MF and accumulate less fluorescent MF, when compared to wild-type strain. Interestingly, the impact on miltefosine susceptibility was higher in amastigote than promastigote forms. Similar findings were observed when *MT* gene of *L. (V.) braziliensis* was overexpressed in the resistant line of *L. (L.) amazonensis*. Finally, we evaluated the gene copy number in others species of subgenus *Viannia* and indicated that *MT* gene copy number varies in *Leishmania* species and that in *Viannia* species this gene is in multiple copies per haploid genome, as we described in *L. (V.) braziliensis*. Taken together, our findings indicated that MT of *L. (V.) braziliensis* is functional, affects miltefosine accumulation and susceptibility in this species revealing that it is essential for the activity of the drug against the parasite. **Supported by:**FAPESP - Processo 2021/00171-6; FAPESP - Processo 2016/21171-6; CNPq - Processo 405235/2021-6 **Keywords:**Leishmania braziliensis;miltefosine transporter;drug resistance.

TB-12 - **The fluoroquinolone besifloxacin is active against *Toxoplasma gondii* proliferation *in vitro***

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Ocular toxoplasmosis (OT) is a disease caused by *Toxoplasma gondii*, a protozoan of the Apicomplexa phylum. Worldwide, TO is the primary cause of posterior uveitis associated to infectious diseases. Currently, the standard treatment for OT involves a combination of sulfadiazine and pyrimethamine, along with corticosteroid administration. However, this therapy is limited to the acute phase of the disease and may lead to hypersensitivity reactions and severe adverse effects. The antimicrobial repositioning strategy has emerged as a promising approach for treating toxoplasmosis, due to the presence of the apicoplast, organelle derived from prokaryotes. Previous works showed this organelle hosts essential metabolic pathways for the survival and replication of *T. gondii* and is sensitive to DNA topoisomerase II inhibitors. Besifloxacin is a fluoroquinolone and the active ingredient of Besivance® eye drops, a medication indicated for the treatment of bacterial conjunctivitis. In this study, we evaluated the effect of BSX against tachyzoites of the RH strain after 48h of infection, in vitro. Additionally, we analyzed its impact on the ultrastructure of the parasite and assessed its cytotoxicity towards the epithelial LLC-MK2 cells and the human fibroblasts HFF treated for 48 hours. The cytotoxicity assays showed that the CC_{50} values for BSX were higher than 120µM and 150 µM, for LLC-Mk2 and HFF, respectively. The antiproliferative assay against the RH strain for 48 hours treatment showed a IC_{50} value lower than 10µM. Transmission electron microscopy analysis of infected LLC-MK2 treated with BSX 40µM, for 48 hours showed parasites with swollen mitochondria, abnormal shaped apicoplast, and the appearance of amylopectin granules in the cytoplasm. Tests with other strains are being carried out. These results indicate for the first time that besifloxacin is active and selective for *Toxoplasma gondii* and is a potential alternative for the treatment of OT. **Supported by:**FAPERJ (E_27/2021US) ; CNPq
Keywords: Apicomplexa;Drug repositioning; Chemotherapy tests.

TB-13 - **Investigating *Leishmania* GSK3 as potential target against visceral leishmaniasis**

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Visceral leishmaniasis is the most severe form of leishmaniasis, fatal if untreated and represents an important health problem. Most cases occur in Brazil, Africa, India and Southeast Asia. It is estimated to occur annually 50,000 to 90,000 new cases worldwide. The current treatment for leishmaniasis is complicated due to high toxicity, limited efficacy, long duration regimes, high costs and increased rate of resistant parasites. Based on that, the identification of novel drugs against leishmaniasis is urgently required. A rational strategy to design new therapeutical drugs should consider the identification of essential targets of parasite. For example, glycogen synthase kinase 3 alpha and beta (GSK3α and GSK3β), essential kinase in *Leishmania* promastigotes. Although not much is known about the role in *Leishmania* biology, the human counterparts are involved in cell proliferation. Human kinases inhibitors are one of the most successful classes of drugs comprising large libraries that can be applied against *Leishmania*. In this work, we evaluated 1,397 kinase inhibitors for activity on both GSK3α and GSK3β in *Leishmania*. To better understand the relationship between compound structure, and biochemical and phenotype activity, we synthesized a series of 35 new analogues of the most promising hit identified from the library screen, a pyrazole carboxamide. Although some of synthesized compounds were potent against *L. infantum* promastigotes (IC_{50} < 1 µM), this activity did not always correlate to GSK3 inhibition in biochemical assays. Despite the high percentage of identity of GSK3 between mammalian and *Leishmania*, we are currently investigating possible ways to mitigate compound toxicity to mice macrophages, such as increasing compound selectivity to *Leishmania* intracellular amastigotes.
Keywords:kinase inhibitor;leishmaniasis treatment;Leishmania infantum.

TB-14 - **The more you look, the more you find: detection of *Crithidia sp* in patients with visceral leishmaniasis in Sergipe, Brazil**

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Visceral leishmaniasis (VL) is a neglected disease caused by *Leishmania infantum* that raises concerns for public health in Brazil. Studies have reported the presence of monoxenous trypanosomatids (*Leptomonas* and *Crithidia*) in VL patients, indicating the necessity of developing detection tools able to discriminate these "unusual" infections. Here, we designed primers for species-specific targets of *L. infantum* and *Crithidia* to be useful for parasite detection through PCR and qPCR. LinJ31_2420 primer pair targets a p-nitrophosphatase gene, whose amplicon is specific for *L. infantum*. LVH60_12060_1F primer pair (for qPCR) targets the catalase gene of *Crithidia*. Crid2.1seq and LVH60_tig001 primers pairs (for PCR) target a hypothetical gene found in the genome of *Crithidia sp* LVH60a parasite (isolated previously from VL patients). DNA was extracted from samples of VL patients, as well as cultured clinical isolates, and used for qPCR/PCR reactions (rx). From 17 bone marrow aspirate (BMVL) samples, *L. infantum* was detected in 10, whereas *Crithidia sp* LVH60a was co-detected in three of them: BMVL1 (~1,056 *L. infantum* parasites/rx and ~32,6787 *Crithidia* parasites/rx), BMVL12 (~8 *L. infantum* parasites/rx and 1,822,285 *Crithidia* parasites/rx) and BMVL60 (~3 *L. infantum* parasites/rx and 3 *Crithidia* parasites/rx). Molecular screening of 62 clinical isolates through PCR revealed that 51 samples (82.25%) were positive for *Crithidia sp* LVH60a and only 11 isolates were positive for *L. infantum.* The use of these primers can help identify and differentiate these parasites in clinical samples, contributing to a better understanding of the pathogenicity of parasites and deepen investigation of recurrent and atypical cases of VL. **Supported by:**FAPESP Scholarship 2021/12464-8; FAPESP grant 2016/20258-0
Keywords: Visceral leishmaniasis;Crithidia-related;Molecular Diagnosis.

TB-15 - **Computer-Aided Drug Design to repurposing new *Leishmania infantum* Ascorbate Peroxidase inhibitors by Structure-Based Virtual Screening**

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Computer-Aided Drug Design (CADD) is an interesting approach that has been used with successful to discovery new chemotherapies opportunities for a large spectrum of diseases. Visceral leishmaniasis is a neglected tropical disease that have a limited therapeutic arsenal that is toxic, expensive and, except for miltefosine, are treated parenterally. In this scenario, it is urgent to develop new safe treatments with less toxicity and orally administered. L. infantum ascorbate peroxidase (LiAsP) is an important enzyme responsible for to protect the parasite from oxidative damage caused by hydrogen peroxide and has been used with an important molecular target to discovery of new drugs. So, the main objective of this work is to reposition new alternatives treatment for visceral leishmaniasis from an FDA-approved drugs library using CADD tools. First, a LiAsP model was obtained by homology modelling by Modeller software from L. major ascorbate peroxidase template (PDB: 3VOB). So, a library with 1425 FDA-approved drugs was tried by Structure-Based Virtual Screening (SBVS) with PyRx software. Next, first 110 ligands were selected to evaluate safe and orally administered drugs using PKCSM server. Then 6 ANVISA (Agência Nacional de Vigilância Sanitária) and RENAME (Relação Nacional de Medicamentos Essenciais) approved drugs were selected to investigate the molecular mechanism of action and the LiAsP ligands complex. All six molecules demonstrated significative interaction with essential active site residues, and it was calculated estimated ΔG (kcal/mol) and Ki (μM) of -7.5, 3.0 (FDA058); -7.3, 4.1 (FDA168); -6.9, 7.5 (FDA252); -6.8, 9.6 (FDA815); -6.4, 18.6 (FDA422); and -6.2, 30.8 (FDA092). Taken together, our data demonstrates that CADD and SBVS can be a useful approach to drug repositioning, and thought of this methodology, it was possible to selected 6 LiAsP potential competitive inhibitors and promising drug candidates for repurposing to visceral leishmaniasis. **Supported by:**FAPERJ; CNPQ; IOC/FIOCRUZ
Keywords: Computer-Aided Drug Design;;Drug repurposing; ;Leishmania infantum .

TB-16 - ***In vivo* and *in vitro* anti-*Leishmania amazonensis* effects of the new compound metalocomplex A3310 of Cobalt**

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Leishmania amazonensis is the main etiological agent of Tegumentary Leishmaniasis in Brazil and represents a serious public health problem. Treatment consists of the use of pentavalent antimonials and amphotericin B (AMB). However, these drugs are toxic and resistant strains aggravat the situation. Studies are needed to find alternatives treatments against ***L. amazonensis***, and the use of metallocomplexes is promising in chemotherapy. In this study, the metallocomplex A3310 (cobalt core), was evaluated ***in vitro*** and ***in vivo*** against ***L. amazonensis*** (strain WHOM/BR/75/Josefa). Promastigotes were treated ***in vitro*** at different concentrations and durations and analyzed by light and electron microscopy and with confocal microscopy after JC-1 labeling. The MTT method was performed to evaluate the effect of A3310 on the LLC-MK2 host cell. ***In vivo*** tests were also performed in Balb/c mice infected with ***L. amazonensis*** intradermally in the ear. Animals were divided into 5 groups: Control; A3310 + artificial skin vehicle (ASV); AMB + ASV; Collagen; and ASV. Subsequently, lesion samples were analyzed by histology. The IC_{50} values were 4.9µM (24h), 3.5µM (48h), 3.8µM (72h) and 3.4µM (96h). Low toxicity to the LLC-MK2 host cell was observed with a CC_{50} of 2mM after 48h of treatment with A3310. Treated promastigotes presented ultrastructural changes as abnormal arrangement of the mitochondrial outer membrane around the kinetoplast. A reduction in mitochondrial membrane potential was detected. Histological analyzes showed a decrease in parasite load and greater uniformity in tissues treated with A3310. The complex presented a low IC_{50} value, affecting an essential organelle for the survival of the parasite, a high CC_{50} value for the host cell, and an interesting lesion regeneration showing that A3310 may be a being promising compound against *L. amazonensis*. **Supported by:**CNPq **Keywords:**Tegumentary Leishmaniasis;Leishmania amazonensis;Metalocomplexes.

TB-17 - **Evaluation of dinuclear Fe(III) coordination compounds with *in vitro* nanomolar activity and ultrastructural change in different forms of *Trypanosoma cruzi***

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Chagas disease is a neglected disease caused by the protozoan *Trypanosoma cruzi*, affecting between 6 and 7 million people worldwide, mainly in Latin America. Current therapy uses Nifurtimox or Benznidazole which generates free radicals and causes severe side effects to patients. Therefore, new compounds against this parasite are needed. Metalocomplexes have already been demonstrated as active against *Leishmania* spp. and *Toxoplasma gondii*, being an interesting alternative against *T. cruzi*. Here we evaluated the *in vitro* effect of two Fe(III) complexes against epimastigotes (Y strain) and amastigotes (Dm28c strain) of *T .cruzi*. We evaluated the cytotoxicity of the compounds in LLC-MK2 host cells using the MTT method. Possible morphofunctional alterations of the parasites were analyzed using transmission electron microscopy and confocal after JC-1 labeling. In epimastigotes, IC_{50} values range from 97 to 110 nM (complex **(1)**) and 104 to 122 nM (complex **(2)**), after 48 and 120 h of treatment, respectively. In amastigotes, IC_{50} values range from 61 to 107 nM (complex **(1)**) and 50 to 173 nM (complex **(2)**), for 72 and 96 h of treatment, respectively. The complexes showed low cytotoxicity for LLC-MK2 yielding impressive selectivity index of 167 for complex **(1)** and 454 for complex **(2)** after 96 h of treatment. Treatment with the complexes in both *T. cruzi* forms resulted in extension of the mitochondria as seen by the abnormal arrangement of their outer membrane around the kinetoplast; epimastigotes had altered reservosomes with abnormal spicules, amastigotes had altered nuclear structure, with heterochromatin concentrated in the nuclear envelope. Treatment with the complexes reduced the mitochondrial membrane potential of the parasite. The complexes were active against both forms of *T. cruzi*, presenting an IC_{50} in the nanomolar range, high selectivity index and affecting essential organelles of the parasite. Therefore, the complexes may act as new prototype drugs against *T. cruzi*.
Supported by:CAPES; FAPERJ **Keywords:**Chagas disease;Metalocomplexes;Trypanosoma cruzi.

TB-18 - **METALLOCOMPLEXES WITH ANTI-*TOXOPLASMA* ACTIVITY**

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Toxoplasmosis is an infection caused by an obligate intracellular parasite, *Toxoplasma gondii*. Infection by this parasite causes severe, potentially fatal symptoms in immunocompromised individuals and fetus of pregnant women. Conventional treatment for toxoplasmosis mainly involves the use of pyrimethamine and/or sodium sulfadiazine (SDZ). However, these drugs have severe side effects justifying the development of new therapeutic alternatives to treat this infection. Recently, metallocomplexes have emerged as a promising class of drugs with the potential to be applied in the chemotherapy of toxoplasmosis. In order to contribute to the development of new compounds with anti-*Toxoplasma* activity, in this study, the mononuclear complexes [Cu(HL1)Cl2] **(1)**, [Fe(HL1)Cl3] **(2)**, [Zn(HL1)Cl2] **(3)**, [Zn(HL1)(SDZ)Cl]. 2H2O **(6)**, and SDZ (positive control) were tested at concentrations of 10 and 20μM in LLC-MK2 host cells infected with *T. gondii* (1:5) for 48h. After that time, all compound inhibited the growth of *T. gondii* in the following order: complex **(1)**, SDZ, complexes **(3)**, **(6)**, and **(2)**. Light microscopy revealed that untreated infected cells had many parasites with normal morphology in the parasitophorous vacuoles. SDZ treatment resulted in round-shaped parasites. However, treatment with complex **(2)** resulted in parasites with degraded forms indicating parasite death. Treatment with the other complexes resulted in parasites with irregularly shape, but a few normal parasites were observed after treatment with complex **(1)**. Therefore, of the complexes investigated, **(2)** was more active, with a greater reduction in the infection rate. These data justify new tests for a better understanding of the mechanism of action of complex **(2)** in cells infected by *T. gondii.* **Supported by:**FAPERJ, CNPq, CAPES
Keywords: Toxoplasmosis;chemotherapy;metallocomplexes.

TB-19 - **Deletion of the lipid droplet protein kinase gene affects the production of lipid droplets, macrophage infectivity, and resistance to trivalent antimony in *Leishmania infantum***

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Lipid droplet protein kinase (LDK) is an enzyme involved in the biogenesis of lipid droplets (LDs), which are organelles involved in various functions related to metabolism and signaling in trypanosomatids. Since LDK function has not been investigated in *Leishmania* spp.*,* we generated *LDK*-knockout *Leishmania infantum* lines using CRISPR/Cas9 to assess its role in this parasite. Our results revealed that *LDK* is not an essential gene for *L. infantum* since its deletion did not interfere with parasite survival. Additionally, *LDK* deletion did not alter the growth of promastigote forms of *LDK*-knockout *L. infantum* lines; however, it resulted in a decrease in LDs in the stationary phase of parasite growth. The transcript levels of serine palmitoyltransferase (SPT), a key enzyme involved in sphingolipid metabolism, were found to be upregulated in *LDK*-knockout *L. infantum* lines, likely to compensate for the absence of LDK and to normalize LD production. In the presence of myriocin, a potent inhibitor of the SPT enzyme and an inducer of LD production, *LDK*-deficient parasites showed a reduction in the abundance of LDs in both the logarithmic and stationary growth phases when compared to the control parasites. Furthermore, infection analysis using THP-1 cells showed that 72 h after infection, the number of infected macrophages and intracellular amastigotes was lower in *LDK*-knockout *L. infantum* lines than in the control parasites. In addition, *LDK*-knockout *L. infantum* lines were 1.5-to 1.7-fold more resistant to trivalent antimony than the control parasites. No alteration in susceptibility to amphotericin B, miltefosine, or menadione was noted in *LDK*-knockout *L. infantum* lines. Collectively, our results suggested that LDK plays an important role in the biogenesis and/or maintenance of LDs in *L. infantum,* resistance to trivalent antimony, and parasitic infectivity. **Supported by:**This investigation received financial support from the following agencies: Programa INOVA FIOCRUZ - Fundação Oswaldo Cruz (VPPCB-007-FIO-18-2-94); Convênio Fiocruz- Institut Pasteur-USP (no grant number); Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG – APQ 02816-21), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 304158/2019-4 and 152027/2022-0). **Keywords:**Leishmania infantum;Lipid droplet protein kinase (LDK);Lipid droplets.

TB-20 - **The role of P2X7 receptor during acute infection with EGS strain.**

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P2X7 receptor belongs to the purinergic receptor family, and ATP is the activator molecule. P2X7 receptor activation takes part in *T. gondii* infection control. The activation of this receptor triggers several intracellular pathways involved in production of inflammatory mediators such as cytokines, chemokines, reactive oxygen species (ROS), lysosomal fusion, and IL-1β secretion in the context of *T. gondii* infection. Most recently, our group showed that P2X7 absence improved tissue damage in Me-49 strain acute infection in the gut, increasing parasite load and inflammatory parameters. The EGS strain was isolated in 1998 from the amniotic fluid of a patient in Minas Gerais – Brazil, and presents a recombinant genotype (I/III). In this work, we investigated the P2X7 receptor contribution during acute infection induced by the EGS strain of *T. gondii.* C57black/6 wild-type mice (WT) and P2X7 receptor knockout ($P2X7^{-/-}$) mice were analyzed 8 days post-infection. The infection induced an increase in morbidity in all infected animals. Infected animals presented a decrease the small intestine and loss of intestinal villus, indicative of inflammation. In order to assess liver damage and dysfunction, plasma aspartate transferase (AST) was evaluated. We observed that EGS strain promoted liver damage independent of the presence of P2X7, despite the pronounced increase in liver weight of $P2X7^{-/-}$ mice. The RT-qPCR assay showed increased parasite load in $P2X7^{-/-}$ mice in comparison whit WT. Besides, we observed up-regulation in the expression of IL-12 and TNF-α in WT-infected mice, and an increase in IFN-γ expression in $P2X7^{-/-}$ mice compared with WT-infected mice. The results indicate that although the infection caused by the EGS strain was severe, the presence of P2X7 receptor was important in controlling of parasite load, contributing to the classic immune response against *T. gondii* during infection.
Supported by:FAPERJ: E-26/202.774/2018; E-26/201.086/2022; SEI-260003/015688/2021 / CNPq 306839/2019-9 **Keywords:**Toxoplasma gondii;Purinergic Receptor;Immunology.

TB-21 - **2'-hydroxyflavanone activity against *Leishmania infantum***

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Visceral leishmaniasis (VL) is the most severe form of Leishmaniasis, in which untreated or late diagnosed cases can lead to death. The therapeutic arsenal is still ineffective, high-cost, highly toxic, and have been reported resistance cases in last years. Among the search for new alternatives for leishmaniasis treatment, studies have demonstrated the leishmanicidal effect of natural products, highlighting flavonoids, a class of plants secondary metabolites with antioxidant and anti-inflammatory properties already described. Previous studies demonstrated *in vitro* and *in vivo* effects of 2'-hidroxiflavanone (2HF), a flavanone known for its activity in tumor cells, against wild-type and antimony-resistant *L. amazonensis.* Considering VL importance and 2HF previous results in Cutaneous Leishmaniasis, this study evaluated 2HF *in vitro* and *in vivo* activity against *L. infantum*. Promastigotes of *L. infantum* were incubated with increasing concentrations of 2HF (0– 96 μM) for 72h. Cell density was obtained by Neubauer chamber counting. 2HF inhibited promastigotes of *L. infantum* in a concentration-dependent manner, with IC_{50} of 8.5 μM. In antiamastigote assay, Balb/c peritoneal macrophages were infected with *L. infantum* promastigotes and treated with increasing concentrations of 2HF (0- 96 μM). 2HF demonstrated a significant decrease in the infection index with an IC_{50} of 3.2 μM. In a murine model of VL, Balb/c mice were infected with *L. infantum* promastigotes. After 21 days of infection, mice were treated orally with 2HF (25, 50 or 100 mg/Kg/day) for 5 days with a 12h/12h scheme and then euthanized. Livers were collected and parasite load analysis was performed by limiting dilution assay (LDA). 2HF reduced the parasite load in the liver in a dose-dependent manner, with a 99% inhibition at 100 mg/kg/day. Taken together, these results indicate 2HF promising effects against *L. infantum*, considering as a possible future candidate for VL treatment.
Supported by:CNPq; CAPES; FAPERJ; IOC/Fiocruz **Keywords:**Leishmaniasis;Chemoterapy;Flavonoids.

TB-22 - **High Resolution Melting Analyses for *Leishmania* species clustering: a method for detecting possible new species**

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Several PCR-based techniques have been applied for identification and phylogenetic studies in *Leishmania*, such as conventional and real-time PCR, DNA sequencing, Restriction Fragment Length Polymorphism (RFLP) and more recently High Resolution Melting analyses (HRM). HRM generates thermodynamic differences in the dissociation profile of PCR products resulting in specific signatures due to differences in DNA sequences. Amongst the targets described for *Leishmania* discrimination, heat-shock protein 70 coding gene (*hsp70*) has proven to be useful in identifying many species from different geographical origins. This gene is conserved across several groups of organisms and its sequence have been used in phylogenetic inferences and species identification. Here we propose a protocol using HRM analyses targeting the *hsp70* sequence for the clustering of more than 50 *Leishmania* strains from a Brazilian collection. Three distinct amplicons were produced in real-time PCR assays and melting temperatures values (Tm) were used as parameter. The tested amplicons showed a greater level of variability for the isolates of the subgenera *L. (Mundinia)* and *L. (Viannia)*. Amplicon 1 separates subgenera *L. (Leishmania)* and *L. (Viannia)* and discriminates *L. (L.) waltoni*, *L. (L.) infantum*, *L. (L.) donovani* and *L. (L.) tropica* from the other members of the subgenus *L. (Leishmania)*. Amplicon 2, a specific target for the subgenus *L. (Viannia)* discriminates *L. (V.) braziliensis* and *L. (V.) naiffi* from the other members. Amplicon 3, despite not grouping taxonomically close species, clustered the species in 5 groups, discriminating species that could not be separated by the other two targets. Furthermore, this method interestingly confirms the individuality of isolates that previously could not be identified at the species level. In conclusion, the protocol described herein is an alternative for identification of *Leishmania*, potentially useful for epidemiological, diagnostic and taxonomic studies. **Supported by:**FAPESP 2018/23512-0
Keywords: Leishmania;Diagnosis;HRM.

TB-23 - **Development of amidoxime derivatives with leishmanicidal activity in *L. amazonensis***

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As a neglected disease, leishmaniasis requires safer and less expensive new oral treatments. Because of this general interest, our project concerns the synthesis of amidoxime derivatives presenting a 4,5-dihydrofuran 3-carboxamide that could offer a new option for the treatment of leishmaniasis. We studied the influence of substituents on antileishmanial activity and focused on monoamidoximes scaffolds. The discovery of its transformation by various enzymes into amide with subsequent release of NO or its reduction to amidines has attracted medicinal chemists to use this group as a potent pharmacophore in the creation of more efficient drugs/prodrugs. Improvement of physicochemical properties such as solubility is also intended. The main objective is to improve the activity without significantly increasing the toxicity. This way, the amidoximes derivatives were designed and synthesized to have different functional groups generating the OSC series. The leishmanicidal activity was determined in promastigotes of *L. amazonensis*. All derivatives were active and showed parasite growth inhibition profile with IC_{50} ranging from 15 to 101.7 µM. It is possible to observe that several substituents were tested in an attempt to improve the leishmanicidal activity as substituents of the heterocyclic type, and mononuclear aromatics such as pyridine and diazines (pyrazine and pyridazine). Of the derivatives tested, derivatives OSC166 (IC_{50} = 17µM), OSC170 (IC_{50} = 16µM) and OSC171 (IC_{50} = 15µM) with pyridine substituents were the most active in the series. Mechanistic studies are desirable to elucidate the pharmacological mechanism of amidoximes and thus understand the observed activity. **Supported by:**CIENTISTA DO NOSSO ESTADO - FAPERJ E-26/202.918/2018 **Keywords:**amidoximes;leishmanicidal activity;L;amazonensis.

TB-24 - **Establishment and validation of a medium-throughput screening method to discover new hits and leads for the treatment of leishmaniasis**

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Leishmaniasis is a public health problem and current treatments have some disadvantages such as a small therapeutic arsenal, high toxicity, and resistance induction, so it is necessary to search for new compounds with new mechanisms of action that can become a drug candidate. In the hit-to-lead process, different types of screening tests can be performed, and intracellular amastigote is considered the gold standard of phenotypic screening assays. Unfortunately, the routine method for quantifying amastigotes depends on exhaustive counting under an optical microscope and on previous experience for data analysis, making the process unfeasible to analyze a large number of compounds. It is known that reporter genes can be a useful tool as they can be used in quantification in phenotypic assays, so this work proposes the establishment and validation of a medium throughput screening methodology using intracellular amastigotes of Leishmania amazonensis transfected with green fluorescent protein (GFP). New assays were carried out for analysis by high content screening (HCS) using intracellular promastigotes and amastigotes of parasites transfected with GFP and macrophages marked with DAPI and phalloidin, allowing obtaining good images of the nuclei and cytoskeleton of infected macrophages. The next step is to test the assay standardization using reference drugs and then perform the first screening of synthetic compounds.
Supported by: Cientistas do Nosso Estado - Faperj processo E26-201.158/2022 e Apoio às Instituições de Ensino e Pesquisa Sediadas no Estado do RJ- Faperj processo E26-210.157/2018
Keywords: Leishmania ;High Content Screening ;Phenotypic Screening .

TB-25 - **Importance of different sizes of polymeric particles loaded with AmB in cutaneous leishmaniasis**

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Treatment of cutaneous leishmaniasis (CL) needs safer and more active formulations especially with reduced number of doses. Implants of polymeric microparticles have been shown to promote local sustained release of amphotericin B (AmB) and to be effective with a single dose, no systemic effect. On the other hand, nano-sized particles are expected to be more easily phagocytosed by infected macrophages. The aim of this study was to maximize the single dose efficacy of AmB-loaded PLGA particles. For that, particles of different size ranges like NanoP, MicroP and MacroP were synthesized by emulsion and solvent evaporation. For biology assays, *Leishmania amazonensis* promastigotes ($2x10^5$/mL) were incubated with different concentrations (0.01-100 µg/mL) of nano-microparticles and AmB free for 72 h at 26 °C and cell viability was assayed by resazurin. When tested for cytotoxicity to bone marrow-derived macrophages (BMDM) –$1x10^5$/well were treated with different concentrations (1.23 – 300 µg/mL) for 48h. Nano-microparticles (0.02-0.04 µg/mL) showed activity similar to free AmB (0.01 µg/mL) against promastigotes. The initial release of AmB already manages to control the effect. According to CC_{50}, all particles were less than 4 fold cytotoxic than free AmB (0.8 µg/mL) in relation to NanoP (4.1 ± 0.6 µg/mL), MicroP (4.6 ± 0.7 µg/mL) and MacroP (3.4 ± 0.5 µg/mL). For in vivo studies, BALB/c mice were infected in the ear with *L.a.* GFP ($2x10^6$). Seven days later treatment was performed intralesionally. (10 µg/ single dose) with diferente particles and free AmB in PBS. Lesion sizes were measured 1x/week. On day 38 of infection, ear parasites were measured by fluorimetric and limiting dilution assays. Of all sizes, MicroP was similarly effective as free AmB, reducing parasitic load by 24% and 14%, respectively. In conclusion, PLGA microparticles loaded with AmB (AmphoDepot®) is a good candidate for single dose treatment of CL.
Supported by: Vale do Rio doce **Keywords:**polymeric particles;amphotericin B;leishmaniasis.

TB-26 - **Characterization of in vitro susceptibility of atypical Brazilian strains of *Toxoplasma gondii* to second-choice drugs in the treatment of toxoplasmosis**

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Toxoplasma gondii is an obligate intracellular protozoan responsible for causing toxoplasmosis. Studies with isolates in South America have shown that the strains circulating in this region differ considerably, both in structure and genetic diversity, from those circulating in Europe and North America. Thus, strains from South America were classified as atypical. This diversity may be associated with the wide geographic distribution, fauna and sexual recombination of clonal strains, which may imply differences in the pattern of susceptibility to treatment. The gold standard treatment for toxoplasmosis consists of the combination of Sulfadiazine (SDZ) and Pyrimethamine (PYR) and alternative medicines such as Sulfamethoxazole (SMX), Trimethoprim (TMP), Clindamycin (CLN) and Atovaquone (ATV). There are reports of adverse effects and treatment failures due to the resistance of the parasite, mainly in Brazil. Therefore, the aim of this study was to study the in vitro susceptibility of these atypical strains (TgCTBr4, TgCTBr11, TgCTBr17 and TgCTBr23) with decreased susceptibility to SDZ and PYR to alternative drugs used in the treatment of toxoplasmosis. For in vitro assays, tests were performed to evaluate the antiproliferative effect of drugs against *T. gondii* in Normal Human Dermal Fibroblast-neo cells. In assays, the TgCTBr4 strain did not respond well to treatment using SDZ and PYR, however, the association of SMT and TMP may be used as they help reduce parasite proliferation. The use of ATV at a higher concentration helped to reduce parasite proliferation in vitro assays for both strains, with the exception of TgCTBr4. In addition, the TgCTBr11, TgCTBr17 and TgCTBr23 strains are susceptible to the use of SDZ and PYR, and these drugs can be used to treat toxoplasmosis in these cases. Additional tests must still be carried out in order to characterize the in vitro susceptibility of atypical strains to the use of CLN.
Supported by: Conselho Nacional de Pesquisa (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) e Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) **Keywords:**Toxoplasma gondii;Atypical strains;Pattern of susceptibility.

TB-27 - **Identification of a small-molecule inhibitor that selectively blocks DNA-binding by *Trypanosoma brucei* Replication Protein A1**

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Replication Protein A (RPA) is a broadly conserved complex comprised of the RPA1, 2 and 3 subunits. RPA protects genome integrity by binding ssDNA during DNA replication and repair. Using structural modeling, we discover a novel inhibitor, JC-229, that targets RPA1 in *Trypanosoma brucei*, the causative parasite of African trypanosomiasis. The inhibitor is highly toxic to *T. brucei* cells, while mildly toxic to human cells. JC-229 treatment mimics the effects of TbRPA1 depletion, including DNA replication inhibition and DNA damage accumulation. In vitro ssDNA-binding assays demonstrate that JC-229 inhibits the activity of TbRPA1, but not the human ortholog. Indeed, despite the high sequence identity with T. cruzi and Leishmania RPA1, JC-229 only impacts the ssDNA-binding activity of TbRPA1. Site-directed mutagenesis confirms that the DNA-Binding Domain A (DBD-A) in TbRPA1 contains a JC-229 binding pocket. Residue Serine 105 determines specific binding and inhibition of TbRPA1 but not T. cruzi and Leishmania RPA1. Our data suggest a path toward developing and testing highly specific inhibitors for the treatment of African trypanosomiasis.
Supported by:NIH, AGENCIA I+D+i **Keywords:**RPA1;drug discovery;DNA replication.

TB-28 - **Study of the stability of mucopenetrant nanosystems containing retinoic acid (RA) for the improvement of an intranasal vaccine against leishmaniasis**

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Leishmaniasis are neglected diseases caused by protozoans of the Leishmania genus, divided into Cutaneous, Mucosal and Visceral forms. To date, no injectable vaccine has shown to be sufficiently effective and safe for human use. Our group has previously shown that oral and nasal tolerogenic immunization with LaAg, which is predominantly Th2 response biasing, protects against leishmaniasis in different animal models of infection. So, our hypothesis is that the mechanism of vaccine protection is induction of immune tolerance to the leishmanial antigens. Since retinol and RA are important in inducing tolerance, RA has great potential as an adjuvant for our vaccine. Previous studies have shown that the use of solid lipid nanoparticles (sNP-RA) as an adjuvant increases the efficacy of LaAg in different models of leishmaniasis. However, sNP-RA contain only 0,1% RA and aren't functionalized to allow a greater mucopenetrant action, so our group developed a safe and innovative formulation consisting of mucopenetrant liposomes encapsulating RA (Lip-PEG-AR). In this work, we evaluated the stability of liposomes in the vaccine formulation under two storage conditions: at room temperature and at 6 °C . To this end, the vaccine was prepared in three different ways: AR1 (LaAg and Lip-PEG-AR were individually resuspended and subsequently mixed), AR2 (LaAg resuspended and then resuspended Lip-PEG-AR with LaAg), AR3 (LaAg and Lip-PEG-AR resuspended together) and their size was analyzed by Dynamic light scattering (DLS). Finally, the DLS results indicated that the liposome remains stable under both storage conditions, and the different mixing methods did not show a significant change in liposome stability. Therefore, our formulation consisting of mucopenetrant liposomes encapsulating RA, for enhancing its adjuvant effect in our LaAg vaccine, is stable under different conditions. **Supported by:**VALE/COPPETEC
Keywords: Leishmanisis;Vaccine;Retinoic acid.

TB-29 - **SAR study of N,N' disubstituted thioureas against *L. amazonensis*.**

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Leishmaniasis is a parasitic disease caused by protozoans of the genus *Leishmania*. According to WHO, in 2021, 99 countries or territories are endemic, still representing a public health problem in underdeveloped countries. Current treatment for leishmaniasis is based on pentavalent antimonials, amphotericin B, miltefosine, and paromomycin. Given the number of drugs available and their limitations, despite significant advances in treating leishmaniasis, it is still necessary to search for new therapies. Thioureas are molecules widely described in the literature, presenting a wide range of biological activities. In the present study, we evaluated 28 new disubstituted thioureas on *L. amazonensis*. We used as a first screening its effects in the promastigote form, where four compounds had an IC_{50} below 50 µM. After that, these molecules were evaluated on intracellular amastigotes and cytotoxicity. Compound 3e demonstrated greater potency with an IC_{50} of 4.9 µM in *L. amazonensis* amastigotes and 80-fold selectivity. New thioureas were synthesized with piperazine for optimizing first-generation thioureas. In this second generation, nine molecules showed an effect on promastigotes of *L. amazonensis* with IC_{50} less than 50 µM and were tested on amastigotes of *L. amazonensis* and cytotoxicity. In intracellular amastigotes, compound 5i showed the highest potency, with an IC_{50} of 1.8 µM and selectivity of approximately 70 times. The most promising compounds from G1 and G2 were tested in *L. infantum* promastigotes. So far, compounds 3e and 5i have obtained IC_{50}s of 30 µM and 4 µM, respectively. This SAR study points to these molecules as promising for developing new drugs for treating leishmaniasis. **Supported by:**Cientista do Nosso Estado - Faperj processo E26-201.158/2022 e Apoio as Instituições de Ensino e Pesquisa Sediadas no Estado do RJ- Faperj processo E26-210.157/2018
Keywords: Leishmania;chemotherapy;Thioureas.

TB-30 - **Identification and characterization of novel proteins with key roles in Toxoplasma gondii's proliferation**

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Toxoplasma gondii is responsible for causing toxoplasmosis, a disease that affects approximately one-third of the global population. Herein, we focus efforts towards understanding the intricate mechanisms that govern the proliferation of this parasite, as its prime mechanism of pathogenesis relies on intracellular growth.
Notably, the centrosome of *T. gondii* plays a pivotal role in these processes, exhibiting distinctive structural attributes. It has become a promising target candidate for new treatments.
Our goal is to identify and characterize centrosomal proteins in *T. gondii* that play essential roles in the parasite's biology. Briefly, we conducted a bioinformatic search for *T. gondii* homologs of centrosomal and basal body proteins with known cellular localization from *Trypanosoma brucei*, a flagellated protozoa. This search yielded 200 protein candidates, many of which are homologs to bona fide centrosomal proteins well characterized in other systems. To validate unknown candidates, we selected ten uncharacterized proteins based on their expression pattern, conservation, and essentiality scores. Using the CRISPR/Cas9 system, we generated knock-in parasites expressing endogenous protein fusions, enabling us to confirm their localization and expression patterns.
Among the candidates, we identified a glycogen synthase kinase, previously named TPK3. We uncoverd that TPK3 displays a punctate localization near the centrosome in a cell-cycle dependent fashion. Moreover, we explored two additional proteins, a casein kinase and a putative flagellar protein whose preliminary characterization places them on the centrosome. We are currently defining their functions and role in cell division. Unraveling the structure and function of the centrosome in *T. gondii* will not only contribute to our understanding of the cell cycle and life cycle of this parasite but will also facilitate the identification of potential therapeutic targets in the future. **Supported by:**PTR (Pasteur Network), MEC (Ministerio Educación y Cultura Uruguay) **Keywords:**centrosome;Toxoplasma gondii;proliferation regulation.

TB-31 - **A novel approach to study epigenetic marks in *Trypanosoma cruzi***

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T. cruzi is the etiologic agent of Chagas disease and has a complex life cycle. Epimastigotes are the replicative form in the vector, trypomastigotes are the infective stage, and amastigotes are a replicative intracellular stage. Proper cell cycle progression requires multiple factors.
Epigenetics could be relevant for gene regulation. Particularly, lysine 76 of histone 3 (H3K76), could be mono, di or tri methylated by the methyltransferases TcDot1a and TcDot1b. It is not known how H3K76 methylation influences cell cycle progression, but deletion of TcDOT1b arrests the cell cycle in G2/M. Additionally, overexpression of Aurora kinase 1 (TcAUK1), affects the same stage.
One of the main problems to study the epigenetic role of H3K76 methylation, or TcAUK1 in cell cycle progression is that transgenic parasites for these proteins had been hard to obtain or maintain. Therefore, we used Flow cytometry that allowed us to identify the differential methylation of H3K76 and to make quantitative analysis using low amounts of antibodies. Remarkably, we have been able to fine-tune the technique to this end. We have used three strain of *T. cruzi*, Dm28c; K98 and Tulahuen that differ by their virulence. We could detect by Flow cytometer that H3K76 could be mono, di and trimethylated, being the last one the most abundant mark in every strain tested. This result is consistent with previous outcomes that showed that H3K76me3 is present in every step of the cell cycle. Additionally, we could detect TcAUK1 in Dm28c, K98 and Tulahuen. This is important because the subcellular location and expression levels of this protein are finely regulated and it is evasive to Western blot detection.
Furthermore, to check the potential connection between TcAUK1 and TcDOT1 activity, we are testing differential H3K76 methylation in parasites that overexpress TcAUK1.
Our findings surpass our study, since this approach will be useful for the whole community working in trypanosome epigenetics. **Supported by:**Proyecto PA19S01. Programa de Cooperación Científico-Tecnológica entre el Ministerio de Ciencia, Tecnología e Innovación de la República Argentina (MINCyT) y ECOS-Sud de Francia. **Keywords:**Trypanosomatids;Flow cytometry;Histone methyltransferases.

TB-32 - **Vitamin D3 as adjuvant of LaAg vaccin in mucosal against leishmaniasis**

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Leishmania amazonensis is the main species that cause the cutaneous leishmaniasis, a neglected disease. It is estimated 700,000 to 1 million new cases of cutaneous leishmaniasis occur annually. There isn't a vaccine approved for human use, but the LaAg (*L. amazonensis* antigen) has been studied for several years as immunizer. The mucosal route vaccine enables an effective response when the LaAg was tested by intranasal route in BALB/c mice and hamsters. Therefore, the goal's study consists to use the LaAg vaccine, and to use vitamin D3 as an adjuvant, by intranasal route, to induce CCR10 expression in the T lymphocytes and induce them to migrate to skin. To evaluate the efficacy of intranasal LaAg vaccine associated with vitamin D3 as an adjuvant in C57BL/6 and BALB/c mice. Was used C57Bl/6 and BALB/c mice. The mice received two intranasal doses of LaAg or Vitamin D3 or LaAg+Vitamin D3 at 7-day intervals, as the control group mice, we used two doses of PBS. After one week of the second dose, the mice were challenged with 2x105 subcutaneously with *L. amazonensis* promastigotes in the hind right footpad or right ear. The lesion size was measured with a dial caliper once a week to see the thickness of the infected footpad or ear. We measured the lesion size in the ear, and vitamin D3 acted as a good adjuvant of LaAg per provided early protection against leishmania in C57BL/6 mice that was vaccinated, and infected. We measured the ear 18/24/48 hours post infection and we see the vaccine was able to induce late hypersensitivity. One day after the second immunization we see the immune response of the popliteal lymph node that showed to reduce the DC levels but enhances activation molecules in Balb/c mice, therefore, LaAg+Vitamin D3 enhances DCs activation. In the intranasal route, the LaAg+vitamin D3 had shown a slower lesion development, and the DCs was more activated than other groups. More experiments are necessary to confirm that vitamin D3 is a great adjuvant. **Supported by:**FAPERJ, CNPq **Keywords:**Leishmaniasis;Vaccine LaAg;Adjuvant Vitamin D3.

TB-33 - **Effect of MSS in vitro and in vivo in Leishmania infantum**

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Leishmaniasis is a neglected tropical disease caused by different species of *Leishmania*. *Leishmania infantum* is responsible for the most severe clinical manifestation, visceral leishmaniasis (VL) and is fatal in over 95% of cases when not treated properly. It is estimated that 50.000–90.000 new cases of VL occur per year. The treatment of leishmaniasis involves a limited drug arsenal and is associated with problems such as therapeutic failure, high toxicity, high costs and the emergence of resistant cases in different parts of the world. Among the search for new alternatives to combat these diseases, drug repositioning stands out. In this scenario, we highlight MSS, a drug currently used in the clinic for the treatment of a nonparasitic disease. This study evaluated the effect of MSS *in vitro* and *in vivo*. Against the intracellular amastigote, MSS (4–280μM) demonstrated an inhibition of the infection index in a concentration-dependent manner after 72 h of treatment and proved to be nontoxic in the macrophage toxicity assay. The IC_{50} value of intracellular amastigotes was 5.49μM, reaching 93.7% inhibition at the highest concentration. Concerning the murine model of visceral leishmaniasis, in the *in vivo* study, two types of treatments were used, short-term and long-term. BALB/c mice were infected with *L. infantum* promastigotes for 7 days and treated with 1,5, 3 or 6 mg/kg/day MSS, 100 mg/kg/day meglumine antimoniate or vehicle, and the animals were euthanized immediately after treatment (short-term) or 18 days later (long-term). MSS was able to decrease the parasite load in the liver and spleen of infected mice compared to the control and meglumine antimoniate-treated groups. Serological toxicology markers were evaluated, and no significant changes were observed, suggesting the absence of liver and kidney toxicity. Taken together, these results suggest that MSS is a possible candidate for leishmaniasis chemotherapy. Obs: MSS real name hidden for possible future patent. **Supported by:**CNPq; CAPES; FAPERJ; IOC/Fiocruz **Keywords:**Leishmaniasis;Repositioning;Chemotherapy.

TB-34 - **Development of a nitrochalcone derivative with leishmanicidal activity in L. amazonensis**

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As a neglected disease, leishmaniasis requires safer and less expensive new oral treatments. Given this general interest, our project concerns the synthesis of chalcone derivative (LCO36) that could offer a new option for treating this disease. Chalcones are part of a class of naturally occurring flavonoids and, like their synthetic counterparts, have been widely used in medicinal chemistry for drug discovery. In the search for substances with leishmanicidal activity, one of the first active chalcones, the 2',6'-dihydroxy-4'-methoxychalcone (DMC) was isolated from the inflorescences of *Piper aduncum* (Piperaceae). DMC strongly inhibited *L. amazonensis* promastigotes and showed efficacy and selectivity against intracellular amastigotes. DMC served as an inspiration for new derivatives that, after changes in the B ring by various substituents and having the nitro group, showed activity when present in position 3, suggesting that chalcones with a NO_2 substituent in the meta position of the B ring would be promising for the development of selective leishmanicidal drugs. These studies led to the design and synthesis of the nitrochalcones NAT22 and its isomer LCO36, the object of this work. The leishmanicidal activity of LCO36 was determined in promastigotes of *L. amazonensis* and showed a parasite growth inhibition profile with an IC_{50} of 2.7 μM, similar to that found previously to NAT22. This result suggests that the isomerism does not interfere with the leishmanicidal activity of this chalcone. **Supported by:**CIENTISTA DO NOSSO ESTADO - FAPERJ E-26/202.918/2018 **Keywords:**nitrochalcone;leishmanicidal activity;L;amazonensis.

TB-35 - **Antileishmanial activity of triazole hybrids based on 4-quinolone-3-carboxamide core**

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The therapeutic arsenal for leishmaniasis has become obsolete, with unacceptable side effects and increasing cases of resistance. Therefore, we aim to search for new compounds to be used as a therapeutic alternative. Regarding developing new leishmanicidal drugs, two chemical classes that arouse interest are the 4-quinolones and the triazoles. Interest in 4-quinolone derivatives as bioactive substances began after the discovery and development of a family of antibiotics containing 4 (1H)-quinolone-3-carboxylic acid. It gained momentum in the 1980s after the insertion of fluorine in position 6 of the quinolone nucleus, originating the second generation of quinolones called fluoroquinolones. Several studies have also demonstrated the leishmanicidal activity of 4-quinolones. Another chemical class widely studied as a leishmanicidal agent are the azoles. Imidazole and triazole antifungals also show high activity inhibiting sterol biosynthesis and growth of *Leishmania* spp and *T. cruzi.* They act on the enzyme C-14 demethylase, causing decreased functional sterols and accumulating several methylated sterols, leading the parasite to death. Thus, from the core 4-quinolone-3-carboxamide (CR-H), seven triazole hybrids were synthesized, generating derivatives substituted by nitro, chlorine and methyl groups, generating the CT series. All the hybrid derivatives were active and showed ICs_{50} ranging from 8.4 to 65.8μM. Among the molecules in the series, CT-H and CT-mCH_3 were the most potent, with an IC_{50} of 8.4 μM in promastigotes of *L. amazonensis*. Comparing the structure-activity relationship, it can be observed that the CT-H molecule, when compared to the CR-H that gave rise to it, differ only in terms of the insertion of the triazole ring. Therefore, it is suggested that the triazole ring may be responsible for the observed activity. The effect of the azole hybrids is being evaluated on the biosynthesis of *L. amazonensis* sterols to elucidate the mechanism of action. **Supported by:**Cientistas do Nosso Estado - Faperj processo E26-201.158/2022 e Apoio às Instituições de Ensino e Pesquisa Sediadas no Estado do RJ- Faperj processo E26-210.157/2018
Keywords: Quinolones;Sterols;Nitroderivatives.

TB-36 - **Investigation of Novel Compounds Against *Trypanosoma cruzi*: Targeting Chemotherapeutic Potential and Drug-Likeness Parameters**

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The investigation of the antiparasitic properties of new compounds is encouraged especially in the context of tropical neglected diseases. One such disease in this category is Chagas disease, caused by the protozoan *Trypanosoma cruzi*, which affects approximately 8 million people worldwide. *T. cruzi* shares similarities with other eukaryotic cells, but its enzymes differ from those found in humans, making them potential targets for chemotherapy. In this work, we investigated the effects of new compounds on their trypanocidal potential against *T. cruzi*, toxicity on LLC-MK_2 cells, ADMET properties and possible cellular targets. To accomplish this, parasites were treated for up to 72 hours and submitted to counting on a flow cytometer. Viability assays were performed by MTS/PMS assay. ADMET and molecular docking analysis were performed by specialized software. Our results demonstrated that most compounds did not reduce host cell viability, and on average, the CC50 value was equal to or greater than 50 µM. Among the evaluated drugs evaluated, some of them inhibited amastigotes proliferation, promoted trypomastigotes lysis and presented CC_{50} and LD_{50} values from 1 to 10 µM. Bioavailability radars indicated that the compounds of interest met most drug-likeness parameters. The compounds showing the most promising results are currently undergoing investigation through molecular docking analysis to assess their affinity for binding to cruzipain. Additionally, we are investigating the antiproliferative and lytic effects on T. cruzi, as well as conducting ultrastructural analysis. Based on these findings, we believe these compounds might be more selective to the parasite and can be explored as promising drugs in further analysis of chemotherapeutic studies against *T. cruzi*. **Supported by:**CNPq, FAPERJ
Keywords: Trypanosoma cruzi;Chemotherapy;Drug-Likeness.

TB-37 - **EFFECTS OF ELECTROPORATION ON *Acanthamoeba Polyphaga*.**

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Introduction: *Acanthamoeba* keratitis (AK) is an eye infection whose treatment can be toxic and ineffective, therefore the possibility of using an electroceutical treatment (electroporation) is being studied. **Objective**: To investigate whether the electroceutical treatment will be able to become unfeasible *Acanthamoeba* cysts and trophozoites in vitro. **Materials and Methods**: In this study, the isolate *A. polyphaga* (ATCC 30461) was used, treated from a corneal scraping from a case of AK in the USA. Trophozoites and cysts were counted in a Neubauer Chamber, the concentration was adjusted to obtain an initial inoculum of 100,000 amoebae/mL in the electroporation buffer. Cysts and trophozoites were exposed to an electric field with intensities of 2000 volts and 2500 volts. The in vitro model was divided into the following groups: cysts and trophozoites controls (without electroporation); group 1 cysts and trophozoites (electroporated at 2000 volts); group 2 cysts and trophozoites (electroporated at 2500 volts). The procedure was performed in duplicate. Permeabilization was analyzed by fluorescence microscopy using propidium iodide (PI) associated with the fluorescence dye 4',6-diamidino-2-phenylindole dihydrochloride (DAPI;1:1000). Images were acquired on a Nikon Eclipse TI-U microscope using an excitation wavelength of 488/617 for PI and 340/488 nm for DAPI, and analyzed using Image J. **Results**: the results obtained demonstrate that permeabilization at 2000 volts is 55% for trophozoites and 55% for cysts ($p < 0.05$); while at 2500 volts it is 59% for trophozoites and 59% for cysts ($p < 0.05$). **Conclusion**: both voltages tested were effective for both cysts and trophozoites, given that the percentages of permeabilization were close, with no statistical significance between them, only with the control groups. Therefore, lower voltages will be tested to reach the same potential obtained, as a future possibility of alternative treatment for AK. **Keywords:**Acanthamoeba;Electroporation;Therapie.

TB-38 - **DRUG DISCOVERY STRATEGIES & TOOLS TO FIND NOVEL COMPOUNDS WITH ANTI-TRYPANOSOMATIDS BIOACTIVITY AND STUDY HOST PATOGENS INTERACTIONS**

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This work summarizes our contributions to the drug discovery against trypanosomatids, principally related to *Leishmania infantum* (Visceral Leishmaniasis, VL) and *Trypanosoma brucei* (Sleeping Sickness, SS) at three levels:

a. Target-based: manual (1) and robotized (HTS, 2) screenings and characterization of inhibitors of trypanothione synthetase (TryS), an essential and druggable enzyme of the redox metabolism, trypanothione synthetase (TryS).

b. Phenotypic-based: HCS on the clinically relevant stages of different trypanosomatids using bioluminescent (3,4) and redox fluorescent reporter (2) cell lines.

Remarkable examples of identified TryS inhibitors are the series N^5-acetamide substituted-3-clorokenpaullones (5) and the compound Ebselen (2) (uncompetitive, allosteric, and allosteric/covalent), with sub-μM/ μM potency, selectivity higher than 10 against mammal cells, and proved on redox metabolism effect *versus L. infantum* and *T. brucei*, respectively.

c. Animal infection models: *in vivo* imaging techniques relying on bioluminescent parasites were employed for the genetic validation of different molecular targets (e.g. G6PDH, SS), to study host pathogen-interactions (e.g. quiescent-like condition, VL), and to test the therapeutic efficacy of hits.

Promptly, these bio-tools (available to the scientific community) and discoveries will allow us to make significant advances in the early phase of drug discovery for these devastating diseases.

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Supported by: Comisión Sectorial de Investigación Científica (CSIC), Universidad de la República Uruguay (UDELAR) (3404); Agencia Nacional de Investigación e Innovación, Uruguay (PhD scholarship code POS_NAC_2013_1_11477); Fondo para la Convergencia Estructural del MERCOSUR (Mercado Comun del Sur) (FOCEM); German Bundesministerium für Bildung und Forschung, Germany (KMUinnovativ 5 - 0315814); Deutsche Forschungsgemeinschaft, Germany (KU 1371/9-1); International Pasteur Network (ACIP 17–2015).
Keywords: Reporter gens;Trypanothione dependent redox metabolism;Anti-trypanosomatids drugs.

TB-39 - **Biochemical and functional characterization of *Leishmania* NAT10 enzyme**

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Leishmania spp. causes leishmaniases, and during its life cycle, the parasite shifts between two hosts and needs to adapt to various environmental conditions to survive. This adaptation could involve changes in gene expression and translation. Recently it was observed that mRNAs can be modified by acetylation in mammalian cells, a modification called N4-acetylcytidine (ac4C), catalyzed by the N-acetyltransferase (NAT10). Depending on the position ac4C is added could greatly impact the stability and/or the translation efficiency of the mRNA. Considering the need for *Leishmania* to adapt to different host environments and the post-transcriptionally gene expression regulation in this organism, we decided to investigate the possible role of ac4C in *L. mexicana* using *Saccharomyces cerevisiae* functional complementation and biochemical activity assays. Initially, we performed several bioinformatic and protein structural analyses to find the *L. mexicana* ortholog NAT10 and identify the key residues involved in the acetyltransferase activity, compared to human and *S. cerevisiae* proteins. To perform complementation in yeast, the native (LmexNAT10-WT) and inactive mutated version (LmexNAT10-MUT) of *L. mexicana* NAT10 gene was cloned into the yeast inducible pYES expression vector and transformed in the haploid wild-type and in a thermosensitive mutant strain of *S. cerevisiae*. The expression of LmexNAT10-WT and LmexNAT10-MUT were validated by western blot. Both versions of LmexNAT10 genes were also cloned into the bacterial expression vector pET28a and the heterologous proteins were obtained and purified for *in vitro* acetyltransferase activity assays. In the next steps, we will use the tools generated to validate the functional conservation of LmexNAT10. **Supported by:**FAPESP 2023/02323-3
Keywords:L; mexicana;Acetylation;NAT-10.

TB-40 - **Evaluate the culture positivity of *Acanthamoeba* in contact lenses, lens cases, lens cases solutions and plungers as potentials sources for *Acanthamoeba* keratitis investigation**

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Purpose: To investigate the culture positivity of *Acanthamoeba* in contact lenses, lens cases, lens cases solutions, and plungers to determine whether these would be potential sources for *Acanthamoeba* investigation in patients suspected of *Acanthamoeba* keratitis.**Methods:**Scleral contact lenses (ScCL), non-scleral contact lenses (NScCL), lens cases, lens cases solutions, plungers, and cornea scrapings collected from patients suspected of *Acanthamoeba* keratitis (AK) underwent culture. The culture was carried out in 1.5% non-nutrient agar with a drop of heat-inactivated *Escherichia coli* (DH5a). Contact lenses and the lens cases and plungers were washed with sterile phosphate-buffered saline (PBS) which was also placed in agar. Any additional solution stored in the case was also cultured. The samples were incubated at 28°C to 30°C for 20 days, and the cultures were checked for positivity through optical microscopy.**Results:**Data of 724 cultures from 279 patients collected between July 1988 and April 2022 were analyzed. From the total, 400 were contact lenses and paraphernalia (338 NScCL, 14 ScCL, 13 lens cases, 29 lens cases solutions, and 6 plungers), and 324 were corneal scraping. We observed positive cultures in 31.4% of NScCL, 35.8% of ScCL, 46.2% of the lens case, 41.4% of the lens cases solutions, 16.7% of plungers, and 26.3% of corneal cultures. Of these, 22.3% were positive for both cornea and contact lenses/paraphernalia; 46.2% were negative for both; 14.7% were negative for contact lenses/paraphernalia and positive for cornea scraping, and 17% were positive for contact lenses/paraphernalia and negative for cornea scraping.**Conclusion:**The present study suggests that the culture of contact lenses and paraphernalia may be helpful to further investigate *Acanthamoeba* keratitis since it revealed a high culture positivity for *Acanthamoeba* and was determinant to detect *Acanthamoeba* in almost 17% of the cornea scraping negative culture.**Keywords:**Acanthamoeba keratitis;Contact Lenses;Paraphernalia.

TB-41 - **Evaluation of Acanthamoeba spp. adherence in scleral contact lenses according to lens shape, surface treatment, and amoeba pathogenicity**

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Purpose: To investigate the adherence of *Acanthamoeba* spp. to the scleral contact lens (ScCL) surface according to lens shape, surface treatment, and strain pathogenicity. **Methods:** Two strains of *A. polyphaga* (CDC:V062 and ATCC 30461) and one clinical isolate obtained from a severe *Acanthamoeba* keratitis, were inoculated onto five contact lenses: one first-generation silicone-hydrogel (Lotrafilcon B; adherence control) with Plasma surface treatment; two ScCL (fluorosilicone acrylate) one with a surface treatment composed of Plasma and the other by Plasma with Hydra-PEG; and two flat lenses (fluorosilicone acrylate) with the same surface treatment of the ScCL respectively. The total of trophozoites adhered to the lens surfaces (initial inoculum of 10^5 trophozoites per lens) was assessed by inverted optical light microscopy after 90-minutes incubation. Possible alterations of the lenses surfaces were evaluated by scanning electron microscopy (SEM). Strain pathogenic profiles were performed by the kinetics of *Acanthamoeba* trophozoite growth and encystment. Clinical strain genotyping was evaluated by Sanger sequencing of 18S rRNA gene. For all statistical tests, a significance level of 5% was considered. **Results:** The three isolates tested adhered more to the surface of the ScCL when compared to the flat lenses, independent of the lens surface treatment (p<0,001). The clinical isolate and the ATCC 30461 exhibited a superior pathogenicity profile when compared to the CDC:V062 and higher adherence (p<0,001) to ScCL and flat lens. Folds were observed on the surface of the lenses tested by SEM. Also, it was noticed that the isolates had a rounded and shrunken appearance on the surface of the flat lens and ScCL and an amoeboid and elongated appearance on the surface of the silicone-hydrogel lens. All the *Acanthamoeba* isolates belonged to the T4 genotype. **Conclusions:** The data suggest that the *Acanthamoeba* pathogenicity and lens shape surface interferes on amoeba adherence. **Supported by:**FAPESP nº 2020/11340-0 **Keywords:**Acanthamoeba keratitis;contact lens;adherence.