## TB2 - **ACTIVITY OF PAROMOMYCIN *IN VITRO* AGAINST BRAZILIAN CLINICAL ISOLATES OF *LEISHMANIA* SPP. AND ITS EFFICACY *IN VIVO* ON TREATMENT OF EXPERIMENTAL TEGUMENTARY LEISHMANIASIS**

COSER, E.M.[*1]; FERREIRA, B.A.[1]; YAMASHIRO-KANASHIRO, E.H.[2]; LINDOSO, J.A.L.[2]; COELHO, A.C.[1]
*1.UNICAMP, MOJI-MIRIM, SP, BRASIL; 2.USP, SÃO PAULO, SP, BRASIL.*
e-mail:elizabethmcoser@hotmail.com

Leishmaniasis is a parasitic disease that affects millions of people worldwide in tropical and subtropical areas. In Brazil, the number of cases of disease has increased in recent years, especially in urban areas. The treatment of leishmaniasis in Brazil is limited to the use of parenteral drugs that are expensive and induce serious side effects. Recently, two drugs were approved as alternatives to the treatment of visceral leishmaniasis in Asia: miltefosine and paromomycin (PM). Although these drugs have not yet been approved for use in Brazil, it is urgent to investigate these drugs as alternative for the treatment of leishmaniasis in Brazil. Here we aim to evaluate the susceptibility to PM *in vitro* of clinical isolates of *Leishmania* spp. from patients with tegumentary leishmaniasis, as well as the effectiveness of PM *in vivo* in a murine experimental model of infection of two species that cause cutaneous disease in Brazil: *L. (Viannia) braziliensis* and *L. (Leishmania) amazonensis*. Typing of 17 clinical isolates by PCR of *hsp*70 gene followed by digestion with the restriction enzyme *Hae*III indicated that species of these clinical isolates are *L. (V.) braziliensis* (9 isolates), *L. (L.) amazonensis* (6 isolates), *L. (V.) guyanensis* (1 isolate) and *L. (V.) shawi* (1 isolate). The $EC_{50}$ values of PM against promastigotes of isolates and reference strains ranged from 4.95 to 205.03 □M, while the EC50 values against intracellular amastigotes ranged 0.54 to 61.9 □M. In vivo studies were performed using BALB/c mice infected with *L. (L.) amazonensis* and treated with doses of 75, 150, 300 and 600 mg/kg/day of PM administered intraperitoneally. Animals treated with dosages of 300 and 600 mg/kg/day presented a significant reduction in lesion size. Our next goal is to evaluate the effectiveness of PM in vivo against *L. (V.) braziliensis.* These results will contribute to evaluate the potential use of PM in the treatment of tegumentary leishmaniasis in Brazil. **Supported by:**FAPESP **Keywords:** Tegumentary leishmaniasis; leishmania amazonensis; paromomycin

## TB3 - **MILTEFOSINE AND PAROMOMYCIN SUSCEPTIBILITY *IN VITRO* OF ISOLATES OF *LEISHMANIA (LEISHMANIA) INFANTUM* FROM DOGS OF THE MUNICIPALITY OF EMBU-GUAçU, BRAZIL**

FERREIRA, B.A.[*1]; COSER, E.M.[1]; OLIVEIRA, V.L.[1]; YAMASHIRO-KANASHIRO, E.H.[2]; ROCHA, M.C.[2]; COTRIM, P.C.[2]; COELHO, A.C.[1]
*1.UNICAMP, HORTOLANDIA, SP, BRASIL; 2.USP, SÃO PAULO, SP, BRASIL.*
e-mail:bianca-alves-ferreira@hotmail.com

Visceral leishmaniasis (VL) is a parasitic disease caused by the protozoan *L. (L.) infantum*. The disease is the most severe clinical form of leishmaniases that can lead to death if it is not treated. In Brazil, about 3,000 new cases of the disease are reported annually, with an increasing number of cases in urban and peri-urban areas. Since VL is zoonotic in Brazil, domestic dogs constitute the main reservoir for the parasite, playing an essential role in transmission of disease to humans. The treatment of VL in Brazil consists in the use of pentavalent antimonials and amphotericin B, drugs that are considered expensive, toxic and that require parenteral administration. In this study, we evaluated the activity of miltefosine (MF) and paromomycin (PM) in vitro against isolates of *L. (L.) infantum* from dogs of the municipality of Embu-Guaçu. These drugs have not yet been approved for the clinical treatment of VL in Brazil, although they are highly effective against VL in Asia caused by *L. (L.) donovani*. Species of the isolates were confirmed by PCR through of amplification of *hsp70* gene followed by digestion with the restriction enzyme *HaeIII* as previously described (Montalvo et al., 2012). A moderate variation in MF susceptibility was found among these isolates in promastigotes and intracellular amastigotes. Similar findings were observed for PM. Considering that these isolates are from the same endemic region, we are interesting to investigate the genetic diversity of these isolates and if they belong or not to the same population. For this aim, we are sequencing the ITS (internal transcribed spacer) region of isolates to evaluate the phylogenetic relationship of them. The results obtained in this study will contribute to evaluate the potential of these drugs against *L. (L.) infantum*, the species responsible for VL in Brazil. **Supported by:**FAPESP **Keywords:** Leishmania infantum; miltefosine; paromomycin

TB5 - **MODULATION OF CELL HEME METABOLISM BY TRYPANOSOMA CRUZI: POTENTIAL ROLE OF COMBINATION THERAPY USING ARTEMISININ AND 5-AMINOLEVULINIC ACID AGAINST AMASTIGOTES**

LECHUGA, G.C.[*1]; DA ROCHA, A.S.B.[2]; BOURGUIGNON, S.C.[2]; PEREIRA, M.C.S.[1]
*1.FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.UNIVERSIDADE FEDERAL FLUMINENSE, NITERÓI, RJ, BRASIL.* e-mail:guilherme.lechuga@yahoo.com.br

Chagas disease a silent neglected tropical disease caused by the flagellate protozoan Trypanosoma cruzi affects social and productive life of millions of people worldwide. The current drugs used to treat this disease have a limited effectiveness and produce many side effects. Therefore, there is an urgent need for new therapeutic targets as well as effective and safe drugs. Heme biosynthesis has been highlighted as a potential drug-target and T. cruzi lacks completely the route for its synthesis, acquiring this essential molecule from the host. However, little is known about the interplay between host-cell and intracellular amastigotes in heme acquisition and storage. We depicted the modulation of cell heme metabolism, by the parasite, treating cells with 5-aminolevulinic acid (5-ALA). T. cruzi-infected cells have higher levels of protoporphyrin IX (PpIX) and amastigotes can import and store PpIX from host cells. Additionally, T. cruzi infection decreases approximately 80% of heme-oxygenase-1 expression in Vero cells and cardiomyocytes. Due to its differential heme metabolism in infected cells, we performed a pharmacological approach combining 5-ALA and the antimalarial drug artemisinin (ART), a molecule that is activated by heme. Low cytotoxicity was observed on Vero cells but intracellular amastigotes were more susceptible to treatments. The combination therapy of ART and sub-lethal doses of 5-ALA (1mM) enhanced approximately 9-fold the trypanocidal activity, reaching an IC50 of 11.4 µM. Activity of compounds were selectively improved against the parasite in serum-free medium, reducing ART IC50 from 87.3 µM to 0.62 µM, reaching better activity than Bz (IC50 = 1.85 µM). In addition, the combination of the compounds reduced the expression of fibronectin and collagen in cardiac cultures. These results indicate a dual role of the combined treatment using ART and 5-ALA, targeting T. cruzi and possibly fibrosis in Chagas disease chronic stages. **Supported by:**Fiocruz, FAPERJ, PAPES VI e CNPq **Keywords:** Trypanosoma cruzi ; artemisinin; heme

TB6 - **THERAPEUTIC POTENTIAL OF PTEROCARPANOQUINONE LQB-118 ON EXPERIMENTAL CHAGAS DISEASE**

BRITO, A.C.S.[*1]; SIQUEIRA, L.M.[1]; SILVA, T.[1]; CUNHA, L.A.C.[1]; PAIVA, M.B.[2]; NEVES, R.H.[1]; NETTO, C.D.[3]; COSTA, P.R.R.[3]; SILVA, S.A.G.[1]
*1.UERJ, RIO DE JANEIRO, RJ, BRASIL; 2.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 3.UFRJ, MACAÉ, RJ, BRASIL.* e-mail:carolinne_brito@hotmail.com

Therapy of Chagas disease is very limited and not effective in the chronic phase. Pterocarpanquinone LQB-118 has shown antitumoral and antileishmanial effects. This study aimed to evaluate the activity of LQB-118 in vitro against *Trypanosoma cruzi* and *in vivo* experimental Chagas disease. Epimastigotes and trypomastigotes of *T. cruzi* (Y strain) were treated with LQB-118 for 96h/27°C or 48h/37°C/5%$CO_2$ and quantified daily by Neubauer chamber. Intracellular amastigotes were treated with LQB-118 for 48h/37°C/5%$CO_2$ and quantified by microscopy. The cytotoxicity of LQB-118 to macrophages was evaluated by MTT method. *In vivo*, Swiss mice infected with $10^3$ blood trypomastigotes intraperitoneally and treated orally with LQB-118 (40 mg/kg/day) for 12 days from 7º day post-infection. We evaluated the parasitemia, survival rates, organ weights, serum biochemical parameters and heart histopathology. LQB-118 was able to inhibit significantly ($p<0.0001$) the epimastigotes growth (99,7%) at 5 µM and $IC_{50}$ in 72 hours was 2 ± 0,07µM. LQB-118 reduce 99,5% ($p<0,0001$) of trypomastigotes motility at 20µM and $IC_{50}$ in 24h was 6 µM and 48h was 3 µM. In intracellular amastigotes, LQB-118 was capable reduce 79,5 % ($p<0,0001$) of parasite number for 48h at 10µM and $IC_{50}$ was estimated at 2,7µM. LQB-118 showed toxicity to macrophages at 10µM ($CC_{50}$ 14,8 ±1,2 µM). *In vivo*, the treatment with LQB-118 reduced the parasitemia by 49,5% ($p<0,02$) and 41,14% from the 11th and 12th dpi, respectively. In animals treated with LQB-118, serum creatine levels were reduced and histological analysis of the heart showed a tendency to reduce amastigote nests. These results show that LQB-118 has activity against *T. cruzi in vitro* and *in vivo* showed therapeutic potential by the oral route. **Supported by:**CAPES **Keywords:** Trypanosoma cruzi; lqb-118; therapeutic

TB7 - **DETECTING COMPOUND TARGET ENGAGEMENT IN LIVING *LEISHMANIA* CELLS**

CATTA-PRETA, C.M.C.*[1]; RAMOS, P.Z.[1]; VASCONCELOS, S.[1]; FANTI, R.C.[1]; RIBOLDI, G.P.[1]; MORETTI, N.S.[2]; VAULLAUD, J.[3]; ROBERS, M.[3]; COUÑAGO, R.M.[1]
*1.SGC-CQMED-UNICAMP, CAMPINAS, SP, BRASIL; 2.UNIFESP, SÃO PAULO, SP, BRASIL; 3.PROMEGA, MADISON, USA.* e-mail:carol.cattapreta@gmail.com

Current therapies for Leishmaniasis are far from ideal. Approved treatments have high toxicity, resistance, and relapse. Modern, target-based drug discovery strategies hold the promise of delivering better, safer medicines for this disease. However, progress in this area has been hampered by the lack of tools for validation of new chemical entities effective against validated molecular targets. Here we will use bioluminescence resonance energy transfer (BRET) to detect compound target engagement and determine its potency and binding kinetics within macrophage-residing *Leishmania*. Our assay is based on NanoBRET technology, which requires a cell-permeable fluorescent tracer (BRET acceptor) that can interact with a molecular target fused to NanoLuc, a small engineered luciferase (BRET donor). Displacement of the fluorescent tracer from the target by a competing ligand disrupts BRET and directly proves target engagement in living cells. As proof of concept, we used a well-known pair of ligand: molecular target - the antibiotic trimethoprim (TMP) and its target Dihydrofolate reductase from Escherichia coli (EcoDHFR). We synthesized fluorescent versions of TMP and showed these could interact with EcoDHFR using purified components in vitro. Further, titration of “dark” TMP competitively disrupted BRET. Similar results were obtained in living HEK293 cells expressing NanoLuc-fused EcoDHFR. Next, NanoLuc-fused EcoDHFR will be expressed in Leishmania for the establishment of target engagement assays in infected macrophages. If successful, we will use this new technology to identify new compounds that can bind to essential *Leishmania* proteins previously identified using genetic methods. We believe this technology will aid the discovery of chemical entities that can bind to validated molecular targets in a biological system. Our tool can also be used to characterize mechanisms of action for new compounds, as well as on lead-optimization campaigns. **Supported by:**PROMEGA; FAPESP; EMBRAPII; CNPq **Keywords:** Drug discovery; target engagement; nanobret

TB8 - **A CHEMICAL BIOLOGY PLATFORM TO VALIDATE GENETICALLY-ESSENTIAL PROTEIN KINASES AS DRUG TARGETS IN *LEISHMANIA***

RAMOS, P.Z.*[1]; CATTA-PRETA, C.M.C.[1]; MOTTRAM, J.C.[2]; COUÑAGO, R.M.[1]
*1.CQMED / SGC - UNICAMP, CAMPINAS, SP, BRASIL; 2.UNIVERSITY OF YORK, YORK, UK.*
e-mail:priscila.zonzini@yahoo.com.br

The leishmaniasis, a group of neglected tropical diseases caused by parasites from the genus *Leishmania*, claims over 20,000 lives and affect ~1 million people per year. Current treatments are not satisfactory – most have severe side effects and require hospitalization. Unfortunately, the development of new drugs to treat leishmaniasis has been hampered by the difficulty to identify valid drug targets. Genetic tools provide valuable but limited information on target essentiality, and cannot inform on target druggability. To overcome these limitations, we will employ a chemical genetic approach (known as “bump and hole”) to investigate if small molecule inhibition of *Leishmania* protein kinases shown essential by genetic methods is a viable therapeutic strategy. This strategy utilizes engineered versions of the target kinases in which a structurally-conserved methionine is replaced by an alanine residue, thus creating a “hole” within the protein ATP-binding site. These engineered proteins are termed analog-sensitive kinases because they can be potently and specifically inhibited with ATP analogs containing a bulky substituent (“bump”) that complements the protein’s enlarged ATP-binding pocket (“hole”). Here we will employ this strategy to chemically-validate five genetically-essential *Leishmania* kinases: KKT2, KKT3, CMGCa, CRK9, and GSK3a. We expect the data obtained in this work to illuminate and support future drug development efforts for leishmaniasis. **Supported by:**CAPES, FAPESP, CNPq, SGC **Keywords:** Drug discovery; therapeutic targets; leishmaniasis

TB9 - **AN OPEN SCIENCE STRUCTURE-BASED DRUG DISCOVERY PROGRAMME TARGETING *LEISHMANIA* AMINOACYL TRNA SYNTHETASES**

DOS REIS, C.V.[*1]; BARREIRO, G.[2]; DA SILVA, R.C.[2]; LAMMOGLIA, J.[3]; DIAS, L.C.[3]; KRATZ, J.[4]; COUÑAGO, R.M.[1]

*1.CQMED/SGC-UNICAMP, CAMPINAS, SP, BRASIL; 2.EUROFARMA, SÃO PAULO, SP, BRASIL; 3.UNICAMP, CAMPINAS, SP, BRASIL; 4.DNDI, RIO DE JANEIRO, RJ, BRASIL.*

e-mail:caio.vinicius.dos.reis@gmail.com

Leishmaniasis are diseases caused by parasites of the genus *Leishmania*. Yearly, these illnesses claim more than 20,000 lives and affect more than 1 million people. Globally, more than a billion people live in endemic areas. Unfortunately, approved treatments for Leishmaniasis have serious drawbacks and the drug discovery pipeline for these diseases is not well-populated. Thus, the prospect of new, safer and more effective medicines against Leishmaniasis is not very promising. Here we started a target-based drug discovery program to find new inhibitors of *Leishmania* Aminoacyl-tRNA synthetases (AaRS). AaRS are enzymes responsible for charging specific tRNAs with their cognate amino acids and have been shown to be essential for a number of organisms, including *Leishmania*. In this project, we cloned, expressed and purified two *Leishmania* AaRSs: LysRS and MetRS. We are currently developing biochemical assays for both enzymes and pursuing co-crystallization of these proteins with candidate ligands. We expect these assays to guide a structure-based drug design programme to investigate the use of AaRS as therapeutic targets against *Leishmania*. **Supported by:**CAPES; Eurofarma; DNDi; FAPESP; Embrapii; CNPq; **Keywords:** Aminoacyl trna synthetases; leishmania; structure-based drug discovery

TB10 - **BIOLOGICAL ACTIVITY OF PYRAZOLE DERIVATIVES AGAINST TRYPANOSOMA CRUZI**

MONTEIRO, M.E.S.[*1]; LECHUGA, G.C.[1]; SOUTO, B.A.[2]; LARA, L.S.[1]; VIGANÓ, M.G.[2]; BOURGUIGNON, S.C.[3]; CALVET, C.M.[1]; OLIVEIRA JUNIOR, F.O.R.[1]; ALVES, C.R.[1]; SILVA, F.S.[1]; DOS SANTOS, M.S.[2]; PEREIRA, M.C.S.[1]

*1.FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.UNIVERSIDADE FEDERAL DE ITAJUBÁ, ITAJUBÁ, MG, BRASIL; 3.UNIVERSIDADE FEDERA FLUMINENSE, NITEROI, RJ, BRASIL.*

e-mail:guilherme.lechuga@yahoo.com.br

Drug therapy for Chagas disease remains a major challenge as potential candidate drugs have failed clinical trials. Currently available drugs have limited efficacy and induce serious side effects. Thus, the discovery of new drugs is urgently needed in the fight against Chagas disease. Here, we synthesized and evaluated the biological effect of pyrazole-imidazoline (1a-i) and pyrazole-tetrahydropyrimidine (2a-i) derivatives against relevant clinical forms of Trypanosoma cruzi. The structure-activity relationship (SAR), drug-target search, physicochemical and ADMET properties of the major active compounds in vitro were also assessed in silico. Pyrazole derivatives showed no toxicity in Vero cells and also no cardiotoxicity. Phenotypic screening revealed two dichlorinated pyrazole-imidazoline derivatives (1c and 1d) with trypanocidal activity higher than that of benznidazole (Bz) against trypomastigotes; these were also the most potent compounds against intracellular amastigotes. Replacement of imidazoline with tetrahydropyrimidine in the pyrazole compounds completely abolished the trypanocidal activity of the series 2(a-i) derivatives. The physicochemical and ADMET properties of the compounds predicted good permeability, good oral bioavailability, no toxicity and mutagenicity of 1c and 1d. Pyrazole nucleus had high frequency hits for cruzipain in drug-target search and structure activity relationship (SAR) analysis of pyrazole-imidazoline derivatives revealed enhanced activity when chlorine atom was inserted in meta-positions of the benzene ring. Additionally, we found evidence that both compounds (1c and 1d) have the potential to interact non-covalently with the active site of cruzipain and also inhibit the cysteine proteinase activity of T. cruzi. Collectively, the data presented here reveal pyrazole derivatives with promise for further optimization in the therapy of Chagas disease. **Supported by:**CNPq, Faperj, Fiocruz e PAPES VI **Keywords:** Pyrazole; chagas disease; chemotherapy

TB11 - **EVALUATING THE CHEMOTHERAPEUTIC POTENTIAL OF NEW CYSTEINE PROTEASES INHIBITORS AGAINST *TRYPANOSOMA CRUZI***

ZUMA, A.A.[*1]; DE SOUZA, W.[1]
*1.UFRJ, RIO DE JANEIRO, RJ, BRASIL.*
e-mail:alinezuma@gmail.com

Chagas disease is caused by the protozoan *Trypanosoma cruzi* and affects about 8 million people around the world. The current available drugs cause several side effects, which encourages the development of new alternatives of treatment. *T. cruzi* presents similar characteristics to other eukaryotic cells. However, its enzymes differ from human ones and therefore they can be used as chemotherapeutic targets. In this work, we evaluated the effects of ten new compounds, which possibly target cysteine proteases. These drugs were evaluated for their trypanocidal potential against *T. cruzi*, the effects on parasite ultrastructure, and toxicity on LLC-MK2 cells. For this, epimastigotes and trypomastigotes were treated for up to 72 hours and submitted to counting on flow cytometer. Viability assays by MTS/PMS and transmission and scanning electron microscopy were also performed. Our results show that epimastigote proliferation was inhibited by all compounds evaluated in this work and they presented $IC_{50}$ values between 500 nM and 10 µM. In order to verify possible ultrastructural modifications, we performed electron microscopy analyzes and distinct cellular alterations were observed, such as: unpacking or higher condensation of nuclear heterochromatin, occurrence of autophagic vacuoles, loss of kDNA topology and atypical cell body shape. Regarding LLC-$MK_2$ viability, four compounds (Fen 1, Fen 2, Fen 12 and Fen 13) were considered less toxic, since their $CC_{50}$ values ranged from 20 to at least 50 µM. Based on these data, these drugs were selected to be tested against trypomastigotes and they were able to decrease the percentage of parasites ($LD_{50}$ from 1 to 10 µM). At the moment, the anti-proliferative effect on intracellular amastigotes are under investigation. Hence, 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 on chemotherapeutic studies against *T. cruzi.* **Supported by:**CNPq e FAPERJ **Keywords:** Trypanosoma cruzi; chemotherapy; cystein proteases

TB12 - **ANTI-LEISHMANIA BRAZILIENSIS GP63 PEPTIDASE ACTIVITY OF PHENANTHROLINE AND ITS METAL-BASED COMPLEXES**

ALBUQUERQUE, R.A.A.C.[*1]; GOMESA, S.M.R.[1]; LIMA, A.K.C.[1]; MCCANN, M.M.[2]; SANTOS, A.L.S.[3]; DUTRA, P.M.L.[1]
*1.UERJ, RIO DE JANEIRO, RJ, BRASIL; 2.NATIONAL UNIVERSITY OF IRELAND, MAYNOOTH, IRLANDA; 3.UFRJ, RIO DE JANEIRO, RJ, BRASIL.*
e-mail:raquelangelicabio@yahoo.com.br

Peptidase inhibitors/chelating agents such as 1,10-phenanthroline and its substituted derivatives, either the metal-free state or as ligands coordinated to transition metals, interfere with crucial functions of several biological systems. In previous works, our group described that L. braziliensis produced gp63 molecules sensible to 1,10-phenanthroline. Herein, we initially studied the cellular distribution of gp63 in a virulent strain of L. braziliensis by biochemical and immunocytochemical analyses. After that, we reported the inhibitory effects of three 1,10-phenanthroline derivative compounds, 1,10-phenanthroline-5,6-dione (phendio), [Cu(phendio)2]and [Ag(phendio)2], on both cellular and extracellular metallopeptidase activities produced by L. braziliensis promastigotes as well as their actions on the parasite viability and on the interaction with murine macrophage cells. The gp63 molecules were detected in several parasite compartments, including cytoplasm, membrane lining the cell body and flagellum, and flagellar pocket. The treatment of L. braziliensis promastigotes for 1 hour with 1,10-phenanthroline and its derivatives resulted in a significant inhibition of cell viability. The pre-treatment of promastigotes with metallopeptidase inhibitors induced a reduction on the expression of surface gp63 as well as a significant reduction on the association index with macrophages. In parallel, the treatment of L. braziliensis-infected macrophages with the 1,10-phenanthroline and its derivatives promoted a powerful reduction on the number of intracellular amastigotes. Collectively, the 1,10-phenanthroline and its metal-based drugs present a good perspective for prospective studies to the development of new anti-L. braziliensis drugs. **Supported by:**FAPERJ **Keywords:** Gp63 peptidase; inhibitors; phenanthroline

TB13 - **ANTI-α-GAL ANTIBODIES TO A SYNTHETIC NEOGLYCOPROTEIN BASED ON LEISHMANIA MAJOR TYPE-II GIPL-3 ACT AS BIOMARKERS OF ACTIVE DISEASE AND CURE IN CUTANEOUS LEISHMANIASIS CAUSED BY LEISHMANIA BRAZILIENSIS**

VIANA, S.M.[*1]; MONTOYA, A.L.[2]; CARVALHO, A.M.[1]; BARRAL, A.M.P.[1]; MALDONADO, R.A.[2]; MICHAEL, K.[3]; DE OLIVEIRA, C.I.[1]; ALMEIDA, I.C.[2]

*1.IGM/FIOCRUZ, SALVADOR, BA, BRASIL; 2.UNIVERSITY OF TEXAS AT EL PASO, EL PASO, USA; 3.NIVERSITY OF TEXAS AT EL PASO, EL PASO, USA.*

e-mail:sayomelo@gmail.com

Cutaneous leishmaniasis (CL) caused by *L. braziliensis* is characterized by a range of clinical presentations, including a localized cutaneous ulcer to mucocutaneous or disseminated disease. Treatment is still based on the use of antimonials and cure is clinically defined as the complete healing of the dermal ulcer. No molecular biomarkers (BMKs) of active disease or cure are available. In *L. major*, highly abundant type-II GIPL-1, -2, and -3 are capped with terminal, nonreducing α-galactofuranosyl (α-Galf) or α-galactopyranosyl (α-Galp) residue (Gal*f*β-R, Galpα(1,3)Gal*f*β-R, and Galpα(1,6)Galpα(1,3)Gal*f*β-R, respectively; where R is the remaining glycosylphosphatidylinositol anchor). These GIPLs are conserved throughout the parasite's life cycle, are highly abundant in the amastigotes and, notably, are highly immunogenic to humans. Based on this premise, we evaluated Type-II GIPL-derived structures against a panel of sera obtained from patients with localized cutaneous (LCL), mucosal (MCL), or disseminated (DCL) leishmaniasis from an area in Brazil endemic for *L. braziliensis*. Patients were treated with Sbv (Glucantime; i.v., 20 mg/kg/d, 20 d). Sera were obtained at the time of diagnosis and following clinical confirmation of cure, and were tested by chemiluminescent ELISA, using as antigens synthetic neoglycoproteins (NGPs) derived from type-II GIPL-1, -2, or -3. We observed that GIPL-3-derived NGP (NGP28b) strongly reacted with sera from LCL patients during the active phase of disease, whereas titers significantly decreased upon lesion healing. In contrast, antibody titers to NGP28b were reduced in MCL patients, regardless of active or cured disease. Reactivity to NGP28b was absent in sera from healthy controls or from Chagas disease patients. Our data indicate that a Type-II GIPL-3-derived α-Gal structure from *L. major* is recognized by sera from patients *L. braziliensis* CL and positive serology to terminal α-Gal residues may act as a BMK of active disease and cure. **Supported by:**CAPES **Keywords:** Cutaneous leishmaniasis; biomarker; gipl

TB14 - ***TRYPANOSOMA CRUZI* INOSITOL PHOSPHORYLCERAMIDE SYNTHASE AS A POTENTIAL DRUG TARGET FOR CHAGAS DISEASE**

ESTEVEZ CASTRO, C.F.[*1]; FERREIRA CHAME, D.[1]; GRECO, R.S.[1]; FERNANDES, H.B.[1]; CARDOSO, M.S.[1]; DENNY, P.W.[2]; TEIXEIRA, S.M.R.[1]

*1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.DURHAM UNIVERSITY, DURHAM, UK.*

e-mail:carlosestevez45@gmail.com

Sphingolipids (SLs) are an essential part of all eukaryotic cellular membranes. Inositol phosphorylceramide (IPC) is an abundant SL present in numerous protozoa but absent in mammals. IPC synthase is an integral membrane protein that is conserved between kinetoplastids such as *Trypanosoma cruzi* and *Leishmania major* and constitutively expressed throughout their cell cycle. Thus, IPC synthase constitutes an ideal target for the development of new treatments for Chagas disease, an illness caused by *T. cruzi* infection, which still lacks an effective treatment. Because inhibitory drugs of the *L. major* IPC synthase were recently identified, the objective of this study is to evaluate their effect on the *T. cruzi* enzyme, named TcIPCS, using two types of genetically modified parasites: *TcIPCS* knockout mutants generated with the CRISPR-Cas9 technology and parasites over-expressing the *TcIPCS* gene. To obtain the *TcIPCS* knockouts, epimastigotes were simultaneously transfected with in vitro synthesized small guide RNAs complexed with recombinant *Staphylococcus aureus* Cas9 and a DNA construct containing the neomycin resistance gene flanked by *TcIPCS* neighboring regions. After neomycin selection, cloning and genotyping, parasites with both alleles disrupted were obtained. To generate *TcIPCS* over-expressing parasites, the pROCKHygro expression vector containing the *TcIPCS* coding region with an HA tag was transfected into epimastigotes. After hygromycin selection, cloned cell lines over-expressing the tagged enzyme were characterized by qPCR and immunofluorescence analysis. *TcIPCS* KO parasites showed a subtle growth defect and decreased metacyclogenesis. Also, *TcIPCS* deletion resulted in decreased in vitro infection capacity, intracellular replication and trypomastigote release. In vitro characterization of parasites overexpressing *TcIPCS* as well as tests using compounds with TcIPCS inhibitory potential on both *TcIPCS* mutant cell lines are underway. **Supported by:**CNPq; FAPEMIG; Global Challenges Research Fund; Global Network for Neglected Tropical Diseases. **Keywords:** Trypanosoma cruzi; inositol phosphorylceramide synthase; crispr-cas9

TB15 - **EFFECTS OF PBN DERIVATIVE ON MITOCHONDRIAL DISFUNCTION IN *TRYPANOSOMA CRUZI***

MACEDO, C.M.[*1]; SARAIVA, F.M.S.[1]; COSTA, D.S.S.[1]; DIAS, A.G.[1]; PAES, M.C.[1]; NOGUEIRA, N.P.A.[1]
*1.UERJ, RIO DE JANEIRO, RJ, BRASIL.*
e-mail:carolmachado28@yahoo.com.br

*Trypanosoma cruzi* is the etiologic agent of Chagas disease (CD). The parasite has a biological cycle with three main evolutionary forms: epimatigotes, trypomastigotes and amastigotes. Among the main characteristics of the parasite is the presence of a unique mitochondrion that undergoes changes in plasticity and function throughout the parasite cycle. Chemotherapy is limited to benznidazole which has low efficacy in chronic phase of CD. Thus, PBN, a nitro compound, has been used to imprison free radicals and demonstrated high pharmacological activity in disease models in which oxidative metabolism features a central role. In this context, the aim of this work was to investigate the effects of a PBN derivative, LQB303, on mitochondrial bioenergetics of the proliferative forms: epimastigotes. We used high resolution respirometry with cells treated or not with the compound, and as a result, we observed an impairment of the mitochondrial physiology of LQB303-treated epimastigotes, when compared to the control group. This can be demonstrated by the reduction in the oxygen consumption rate of the parasites in the routine (endogenous oxygen consumption) in 66%, as well as a reduction of the proton leak (proton leakage by inhibition of ATP synthase) in 70% and ETS (maximum capacity of the electron transport system) in 63%, but ROX (non-mitochondrial residual respiration) showed no difference. We also evaluated the production of reactive oxygen species (ROS) by flow cytometry and observed that in a concentration ten times higher than the IC50, there was an increase in ROS when compared to the control. In summary, our results suggest that the induction of changes in mitochondrial physiology leads to a deficiency in oxygen consumption by these parasites, which may contribute to a decrease in *T. cruzi* proliferation by depletion of ATP production. **Supported by:**FAPERJ, CNPq, INCT-EM **Keywords:** Mitochondrial physiology; chemotherapy; trypanosoma cruzi

TB16 - ***TOXOPLASMA GONDII* INFECTION REDUCED IN LLC-MK2 CELLS AFTER *IN VITRO* MELATONIN TREATMENT**

MACHADO, N.I.[*1]; DE SOUZA, W.[2]; DAMATTA, R.A.[1]; SEABRA, S.H.[3]
*1.UENF, CAMPOS DOS GOYTACAZES, RJ, BRASIL; 2.UFRJ, RIO DE JANEIRO, RJ, BRASIL; 3.UEZO, RIO DE JANEIRO, RJ, BRASIL.*
e-mail:nayinocencio90@gmail.com

Toxoplasmosis is an important disease caused by the obligate intracellular parasite *Toxoplasma gondii.* The current treatment for this disease is based on drug administration that causes serious side effects, especially in immunocompromised patients and pregnant women. New researches are essential to look for new therapies that can be administered without risk to the patient throughout the treatment. Melatonin is a hormone responsible for regulating the circadian cycle in vertebrates and is produced by the pineal gland. The most recent studies show melatonin as a possible therapy for other parasites such as *Leishmania* spp, *Trypanosoma cruzi* and *Plasmodium* spp. For *T. gondii*, a few *in vivo* studies show that melatonin can activate cellular immunity during the infection. Thus, the melatonin effect was study in LLC-MK2 host cells infected with *T. gondii* for 24 h, 48 h and 6 days. A significantly reduced infection rate was observed after 6 days of treatment. Withdrawal of melatonin after treatment and further culture showed an irreversible effect. Scanning and transmission electron microscopy analysis revealed parasite with altered shape as well as plasma membrane rupture after treatment with melatonin. Some melatonin treated parasites were positive for apoptosis cell death. These data indicate that melatonin was able to reduce *T. gondii* infection. In conclusion, these studies show that melatonin may become a possible anti-*Toxoplasma gondii* therapy. **Supported by:**FAPERJ, CNPq, UENF, UFRJ, UEZO **Keywords:** Antiproliferative activity; melatonin; toxoplasma gondii

## TB17 - **TOLL-LIKE RECEPTOR AGONISTS KILL *LEISHMANIA AMAZONENSIS* THROUGH MACROPHAGE ACTIVATION**

GRANATO, J.T.[*1]; SALUNK, D.B.[2]; DIB, P.R.B.[1]; MACEDO, G.C.[1]; COIMBRA, E.S.[1]
*1.UFJF, JUIZ DE FORA, MG, BRASIL; 2.UNIVERSIDADE DE PANJAB, CHANDIGARH, ÍNDIA.*
e-mail:julianatgranato@hotmail.com

Leishmaniasis is caused by protozoan parasites of the genus *Leishmania* spp. The mammalian host houses the obligate intracellular form of the parasite.Thus, the profile of immune response plays an important role in the clinical development of the disease and the effectiveness of treatment. Toll-like receptors (TLR) are one of the key factors in developing an adequate immune response against the parasite. In this sense, TLR agonists can be a tool in the treatment of leishmaniasis. In this work, the antileishmanial activity of the two TLR agonists named PU-TLR-1 and PU-TLR-5 was evaluated. In addition, immunological assays to verify the production of key cytokines for parasite destruction were performed. The cytotoxicity of TLR agonists was determined on peritoneal macrophages, by the MTT colorimetric method, after 72h of treatment. Antileishmanial activity was determined in L. amazonensis GFP-infected macrophages after 72h of treatment. Cytokine production (IL-6, IL-12 and TNF-α), nitric oxide (NO) and reactive oxygen species (ROS) were determined in infected and uninfected macrophages by ELISA, Griess reagent and H2DCFDA techniques, respectively. Both TLR agonists was effective against *L. amazonensis*, but PU-TLR-5 agonist was the most potent and most selective compound (IC50 = 5.93 uM and SI = 2.81). This compound was able to increase the production of proinflammatory cytokines IL-12, IL-6, TNF-α as well as NO and ROS levels in infected and uninfected macrophages compared to unstimulated control. The data show that PU-TLR-5 presents immunomodulatory characteristics capable of activate macrophages to produce proinflammatory cytokines and these results demonstrate the potential of TLR agonists for the treatment of leishmaniasis. **Supported by:**CNPq, Fapemig, UFJF and CAPES **Keywords:** Leishmania amazonensis; tlr agonists; cytokines

## TB18 - **IN VITRO ACTIVITY OF QUERCETIN FLAVONOID ON *TRYPANOSOMA CRUZI***

CUNHA, L.A.C.[*1]; SERAFIM, A.S.[1]; BRITO, A.C.S.[1]; SANTOS, R.F.[1]; COSTA, S.S.[2]; SILVA, S.A.G.[1]
*1.UERJ, RIO DE JANEIRO, RJ, BRASIL; 2.UFRJ, RIO DE JANEIRO, RJ, BRASIL.*
e-mail:luan_alm@hotmail.com

Chagas disease is one of the most neglected diseases in the world and treatment with benzonidazole or nifurtimox is only limited to the acute phase of the disease. Quercetin is a flavonoid present in various plant species and has activity against *Leishmania spp* and *Trypanosoma brucei*, however, few studies have been performed on *T. cruzi*. The present study aimed to investigate the in vitro action of quercetin on the evolutionary forms of *Trypanosoma cruzi*. Epimastigote forms (clone Dm28c) were incubated with quercetin (0-330 µM) for 96h / 27 ° C and counted daily in a Neubauer chamber. For assays with intracellular amastigote forms, peritoneal macrophage monolayers were infected with Y strain (1 parasite / cell) trypomastigotes and incubated with quercetin (0-330 µM) for 48h / 37 ° C / 5% CO2. The possible cytotoxicity of quercetin to mammalian cells was investigated using VERO cells and murine peritoneal macrophages, which were treated with flavonoids for 48h and viability assessed by the MTT reduction assay. Quercetin showed no activity on epimastigote growth in culture at any of the concentrations tested. However, in amastigote forms, quercetin showed significant activity and IC50 estimated at 160 µM. At the tested concentrations, quercetin did not alter the viability of VERO cells or murine peritoneal macrophages (CC50 > 660 µM). These data show that quercetin exhibits inhibitory activity on intracellular amastigote forms of *T. cruzi*, with low toxicity to mammalian cells. Trials on trypomastigote forms are underway. **Supported by:**CNPq **Keywords:** T. cruzi; quercetin; mammalian cells

TB19 - **THE ROLE OF CIRCULATING EXTRACELLULAR VESICLES IN PATIENTS WITH CHRONIC CHAGAS DISEASE**

MADEIRA, R.P.*[1]; BUCK, P.C.[2]; FERNANDES, F.[2]; RAMIRES, F.[2]; CARDOSO, J.N.[2]; IANNI, B.M.[2]; MADY, C.[2]; TORRECILHAS, A.C.T.[1]
*1.UNIFESP, DIADEMA, SP, BRASIL; 2.USP, SAO PAULO, SP, BRASIL.*
e-mail:rafaelpedromadeira@gmail.com

Chagas Disease is a neglected tropical disease (NTD) caused by the flagellated protozoan Trypanosoma cruzi (T. cruzi) and is a major public health problem initially in Latin America, but now expanding over the globe. Since the decade of 1970, many groups have showed that eukaryotic cells release extracellular vesicles (EVs) and EVs have an important role in intercellular communication both in physiological and pathological conditions. As the interaction between host and pathogen is crucial in the establishment of disease and knowing the importance of signalling mediated by EVs. Our study proposes to characterize and compare the circulating EVs isolate from serum of the Chronic Chagas Disease patients and donors. Peripheral blood was collected from patients and donors, followed to separate plasma and enrich the EVs total shedding. The plasma was subjected to ultracentrifugation to isolate the EVs, which then had their size and concentration characterized by Nanoparticle Tracking Analysis (NTA). The EVs were incubated with THP-1 cells, that after interaction had their supernatant analysed by ELISA to cytokines detection. The amount of circulating EVs in chronic Chagas patients lower than compare with donors, as for their size there was no relevant difference. In relation to their ability to induce cytokine production, the chronic Chagas patients EVs were able to induce a higher production of TNF-alpha. In donors, circulating EVs have a more pro-inflammatory and decrease concentration of the chronic stage and may be related to a diminished immune response in order to allow the parasite persistence in the host. As for the increased TNF-alpha production, it may be a host response to the parasite, but one unable to control and resolve the infection. **Supported by:**FAPESP, CAPES **Keywords:** Extracellular vesicles; immune response; chagas disease

TB20 - **CHARACTERIZATION OF CIRCULATING EXTRACELLULAR VESICLES ISOLATE FROM SERUM OF THE IMMUNOSUPPRESSED CHRONIC CHAGAS PATIENTS**

MADEIRA, R.P.*[1]; DE BARROS, L.A.[1]; PEREIRA CHIOCCOLA, V.L.[2]; TORRECILHAS, A.C.T.[1]
*1.UNIFESP, DIADEMA, SP, BRASIL; 2.IAL, SAO PAULO, SP, BRASIL.*
e-mail:rafaelpedromadeira@gmail.com

The evaluation of extracellular vesicles (EVs) has been increasingly reported in the past years as their role in homeostasis and disease where they mediate intercellular communication has been well characterized. The parasite is able to escape the host immune system and establish itself as a chronic infection leading to major cardiac problems mainly. Being the immunosuppressed state a factor that alters how the body functions, in special in how it deals with external agents as pathogens. The aim of this study was to characterize the size and concentration of circulating EVs isolate from serum of the immunosuppressed chronic Chagas patients that had reactivation of the disease. Peripheral blood was collected from patients and centrifuged at 1850 xg for 15 min to separate the plasma, which was then ultracentrifuged at 100,000 xg for 1 h to purification of EVs. The isolated material was then characterized by Nanoparticle Tracking Analysis (NTA). Mean particle size and dispersion measures showed no difference when patients were grouped by sex or age, but when measuring concentration of particles/mL, data showed an increase with age. When co-morbidity with other diseases was assessed, we observed variation both in mean particle size and dispersion and particle concentration. In summary, age and co-morbidities in immunosuppressed patients with reactivation of Chagas disease led to variable particle size and concentration data which might correlate to altered host response to both primary infection and co-morbidities. **Supported by:**FAPESP, CAPES **Keywords:** Extracellular vesicles; immunosuppression; chagas disease

TB22 - **ANTILEISHMANIAL EFFECT AND MODE OF ACTION OF NEW HYDRAZONES DERIVATIVES IN *LEISHMANIA AMAZONENIS***

GRANATO, J.T.[*1]; PINHEIRO, A.C.[2]; TERROR, M.S.[1]; SOUZA, M.V.N.[2]; COIMBRA, E.S.[1]
*1.UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA, MG, BRASIL; 2.FUNDAÇÃO OSWALDO CRUZ, INSTITUTO DE TECNOLOGIA EM FÁRMACOS-FAR MANGUINHOS, RIO DE JANEIRO, RJ, BRASIL.*
e-mail:julianatgranato@hotmail.com

Leishmaniasis is a neglected diseases distributed in tropical and subtropical countries around the world affecting approximately 1 million people. Chemotherapy is the main tool for the treatment of leishmaniasis. However, high toxicity and adverse effects limit available medications. Hydrazones are a functional groups of molecules known for antitumor and antiparasitic activities, including antiplasmodial and antileishmanial. So, the antileishmanial effect of new hydrazone derivatives were evluated, as well the mode of action of the best compound in *Leishmania amazonensis*. Antipromastigote test and cytotoxicity on peritoneal macrophages were performed using the MTT colorimetric method after 72h of treatment. The effect on intracellular amastigotes was determined in *L. amazonensis*-GFP after 72h of treatment.The effect of the best antileishmanial compound, 2f on mitochondria was verified through mitochondrial membrane potential (Δψm) and reactive oxygen species (ROS) production using Mitotracker and H2DCFDA, respectively. The integrity of the parasite plasma membrane (PPM) was evaluated using propidium iodide (PI). Regarding antipromastigote activity, all compounds were active, but the compounds 2b, 2f and 2e stand out for having the lowest IC50 values (7.95; 9.66 and 10.52 µM, respectively). The compounds, in general, showed low cytotoxicity in macrophages, but the 2b stands out for not being cytotoxic (CC50> 150 µM). Regarding antimastigote activity, compounds 2f, 2e and 2b, were the most active (IC50= 4.07; 10.05 and 14.51µM, respectively). Promastigotes treated with 2f at the highest concentration (19.32 µM) induces significant reduction (30.27%) in Δψm compared to untreated control. Regarding to ROS, 2f (9.66 and 19.32µM) induced a increase (111% and 88.13%, respectively) in ROS production. Compound 2f does not alter the integrity of the PPM. The data indicate that the induction of oxidative stress may be one of the major contributing factors to parasite death. **Supported by:**CNPq, Fapemig, UFJF and CAPES **Keywords:** Leishmania amazonensis; hydrazones; mitochondria

TB23 - **EFFECT OF SERINE PROTEASES INHIBITORS AGAINST *LEISHMANIA* SPECIES**

MACHADO, P.A.[*1]; GOMES, P.S.[1]; COIMBRA, E.S.[2]; GUEDES, H.L.M.[1]
*1.UFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.UFJF, JUIZ DE FORA, MG, BRASIL.*
e-mail:patriciamachado12@yahoo.com.br

Introduction and objectives: Leishmaniasis is caused by *Leishmania* parasites and causes 26,000 to 65,000 annual deaths. The control is based on chemotherapy, however the drugs available are toxic and parasite resistance has been reported. Therefore, the search for new compounds with antileishmanial effect and low toxicity is necessary. Proteases are virulence factors in *Leishmania* and consequently emerge as potential new drug targets in these parasites. So, this study aimed to evaluate the antileishmanial effect of serine protease inhibitors, named C1 and C2, on *L. amazonensis* and *L. infantum*. Material and methods: The effect of the compounds was evaluated against promastigotes and amastigotes, and cytotoxicity in peritoneal macrophages was also determined. Anti-promastigote test and cytotoxicity were performed using the MTT method after 72 h of treatment. Anti-amastigote test was realized in macrophages infected with amastigotes after 72 h incubation period. The amastigotes viability was analyzed by measuring the fluorescence intensity of amastigotes for *L. amazonensis* (using a strain transfected with green fluorescence protein) or intracellular parasites counting for *L. infantum*. $CC_{50}$ and $IC_{50}$ values were calculated. Results: Compounds C1 and C2 showed low toxicity against host cells, with $CC_{50}$ values of 189.07 and 114.94 µM, respectively. Against promastigotes, C1 ($IC_{50}$ = 2.02 µM for *L. amazonensis* and $IC_{50}$ = 2.78 µM for *L. infantum*) was more active than C2 ($IC_{50}$ = 59.23 µM for *L. amazonensis* and $IC_{50}$ > 200.00 µM for *L. infantum*) for both species. Against intracellular amastigotes, there was variation between species. In *L. amazonensis*, C2 ($IC_{50}$ = 80.24 µM) showed a best result when compared with C1 ($IC_{50}$ > 200.00 µM), while on *L. infantum*, C1 ($IC_{50}$ = 8.93 µM) was more active than C2 ($IC_{50}$ = 32.75 µM). Conclusion: These results highlight the antileishmanial potential of protease inhibitors, principally against *L. infantum*. **Supported by:**FAPERJ, CNPq, CAPES **Keywords:** Serine proteases; leishmania amazonensis; leishmania infantum

TB24 - **EFFECT OF EPIGALLOCATECHIN-3-GALLATE IN *LEISHMANIA INFANTUM* PROMASTIGOTES: PRODUCTION OF HYDROGEN PEROXIDE AS A MECHANISM OF ACTION.**

DE OLIVEIRA, C.B.[*1]; INACIO, J.D.F.[1]; ALMEIDA-AMARAL, E.E.[1]
*1.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL.*
e-mail:carinebarboosa2@gmail.com

Leishmaniasis is a neglected tropical disease caused by species of protozoan parasites of the genus *Leishmania*. In addition to generating side effects and resistance, treatment for leishmaniasis remains mostly ineffective and expensive and has a long duration, making natural products are an important alternative for treatment of the disease. Epigallocatechin-3-gallate (EGCG) is the most abundant flavonoid constituent of green tea, and it has been reported to have antiparasitic activity in vitro against *L. amazonensis* and *L. braziliensis*. The aim of this study is to demonstrate the effect of EGCG on *L. infantum* promastigotes and possible mechanism of action. *L. infantum* promastigotes were incubated with EGCG for 72h and the cell viability was estimated by Alamar Blue assay. EGCG inhibited promastigote proliferation in a dose-dependent manner. To evaluate the mechanism of action of the EGCG, *L. infantum* promastigotes were incubated for 72h with increasing concentrations of EGCG and production of $H_2O_2$ was measured using Amplex Red Reagent. Promastigotes demonstrated a dose-dependent production of $H_2O_2$, reaching an increase of 7 folds when compared to control. Additionally, we observed a linear correlation between the inhibition of promastigotes proliferation and the $H_2O_2$ production ($r^2$=0.82). Thus, in order to better study the mechanism of action, EGCG was associated with peg-catalase. *L. infantum* promastigotes were incubated with increasing concentrations of EGCG in absence or in the presence of peg-catalase. As expected, promastigotes proliferation inhibition was observed in absence of catalase. However, when EGCG was incubated with catalase, this enzyme was able to protect the promastigotes from oxidative stress caused by EGCG. These results demonstrate that EGCG is a promising compound for the visceral leishmaniasis treatment, being able to inhibit the proliferation of *L. infantum* promastigotes by an increasing of $H_2O_2$ production leading to death of these parasites **Supported by:**FAPERJ, CAPES, CNPq and FIOCRUZ
**Keywords:** Leishmania infantum; epigallocatechin-3-gallate; hydrogen peroxide

TB25 - **DEVELOPMENT AND VALIDATION OF A SCREENING METHOD TO DISCOVER NEW "HITS/LEADS" FOR LEISHMANIASIS TREATMENT**

SANTOS-SILVA, K.E.[*1]; ANDRADE-NETO, V.V.[1]; SOARES, R.P.[2]; TORRES-SANTOS, E.C.[1]
*1.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.INSTITUTO RENE RACHOU, BELO HORIZONTE, MG, BRASIL.* e-mail:karine.ess@hotmail.com

Fluorescent reporter genes are promising tools for new drug screening models, since the conventional assays are exhaustive and error prone. In this work, we developed and validated a phenotypic assay using promastigotes and amastigotes of Leishmania amazonensis transfected with integration plasmid containing red fluorescent protein (RFP). The leishmanicidal activity of miltefosine in L. amazonensis WT (LaWT) and in L. amazonensis RFP (LaRFP) promastigotes was evaluated to compare the standard assay by resazurin addition and the fluorimetric RFP assay in 96 wells plate (ex. 560 nm, em. 620 nm), obtaining an IC50 of 14.1 µM (10.7 - 18.4 µM) for LaRFP and 20.9 µM (20.0-21.9 µM) for LaWT. In the next step, the antiamastigote activity was evaluated using LaRFP-infected macrophages by flow cytometry. The higher the concentration of miltefosine, the lower the number of amastigotes per infected cell and lower the average fluorescence intensity. From this, it was possible to calculate the IC50 value obtained for the treatment with miltefosine, which was 3,7 µM (0,5 – 0,6 µM). Antipromastigote activity was also evaluated for new drug candidates of compound series containing triazoloquinolone core (TZQs), initially in LaWT. Subsequently, the leishmanicidal activity of the most potent molecule, TZQ19, that showed an IC50 of 13,3 µM (12,3 – 14,3 µM), was evaluated in LaRFP promastigotes, with and without the addition of resazurin, obtaining concordant IC50 values, of 24,76 µM (22,0 – 27,9) and 23,7 µM (16,3 – 34,5), respectively, validating the method on this molecule. Therefore, we can conclude that the use of fluorescent reporter genes emerges as a potential method for drug screening. **Supported by:**CNPq **Keywords:** Leishmania amazonensis; red fluorescent protein; fluorescence

TB26 - **DISRUPTION OF KHARON1 IN *LEISHMANIA INFANTUM* AMASTIGOTES DOWNREGULATES MRNA THAT ENCODES FOR CELL DIVISION/CYTOSKELETON-ASSOCIATED PROTEINS CENTRIN1, KMP-11 AND SAS-6**

ROSA-TEIJEIRO, C.[*1]; MOREIRA, P.O.L.[2]; FERNÁNDEZ-PRADA, C.[1]; MONTE NETO, R.L.[2]
*1.FACULTÉ DE MÉDECINE VÉTÉRINAIRE - UNIVERSITÉ DE MONTRÉAL, ST-HYACINTHE, CANADÁ; 2.INSTITUTO RENÉ RACHOU - FIOCRUZ MINAS, BELO HORIZONTE, MG, BRASIL.*
e-mail:rubensdomonte@gmail.com

The disruption of kharon1 gene in *Leishmania* spp. leads to live-attenuated amastigotes, imparing cell cycle progression due to cytokinesis defect and the parasites are unable to maintain infection. This could be considered an alternative for vaccine development. Furthermore, kharon1 knock-out *Leishmania infantum* can be used to better understand the molecular basis of cell division, a field poorly explored in these parasites. Here we selected *L. infantum* kharon1 null mutants (KOLikh1) and evaluate by qRT-PCR the modulation of transcripts that encode for six cell division/cytoskeleton-associated proteins: Centrin1, KKT (kinetochore), SAS-6 (spindle assembly abnormal protein6/basal body cartwheel protein-6), KMP-11, Sbf-1 (spindle body protein) and AIRK (Aurora I-related kinase). In KOLikh1, Centrin1, SAS-6 and KMP-11 were enormously downregulated 30.6, 18.6 and 11.6 folds, respectively. Suggesting that during cell division kharon1 play a role dependent of these factors which are probably orchestrating cell division dynamics in *L. infantum*. Whether they act as a protein complex or regulating (direct or indirectly) cell division is yet to be described. The other targets analyzed were slightly or non-modulated in KOLikh1. Curiously, when disrupted, KH1, centrin1 or KMP-11 (independently), leads to a similar phenotype of live-attenuated multinucleated parasites. Further analyses are being performed to evaluate the role of kharon1 in *Leishmania* cell division. The basic findings here can be applied to better understand the mechanisms of chromosomal segregation which is very particular in *Leishmania* parasites and can be used as a model of mosaic aneuploidy. Additionally, these proteins can be studied as drug targets, supporting the development of alternative drugs for tackling leishmaniasis. **Supported by:**Fapemig, CAPES, CNPq **Keywords:** Leishmania; cell division; kharon1

TB27 - **STUDY OF THE EFFECTS OF HISTONE DEACETYLASE INHIBITORS ON *TOXOPLASMA GONDII***

ARAUJO-SILVA, C.A.[*1]; GUIMARÃES, J.M.[2]; DE SOUZA, W.[1]; MARTINS-DUARTE, E.S.[3]; VOMMARO, R.C.[1]
*1.UFRJ - IBCCF, RIO DE JANEIRO, RJ, BRASIL; 2.UFF, RIO DE JANEIRO, RJ, BRASIL; 3.UFMG, BELO HORIZONTE, MG, BRASIL.*
e-mail:cahh.araujo@gmail.com

Toxoplasmosis is a cosmopolitan zoonosis of great medical importance caused by the obligate intracellular protozoan *Toxoplasma gondii*. *T. gondii* infection can cause uveitis, congenital diseases and encephalitis in immunocompromised individuals. Treatment of toxoplasmosis is restricted to some medications, which are commonly related to various side effects. In addition, the drugs available are effective only against the acute phase of the disease and do not promote parasitological cure of the chronic stage of the disease. Thus, the discovery of new compounds for the treatment of toxoplasmosis is necessary. Recent studies show that histone deacetylase inhibitors are potential chemotherapeutic agents for parasitic infections. The activity of two histone deacetylase inhibitors, Tubastatin A (TST) and Suberoylanilide Hydroxamic Acid (SAHA) were evaluated on *T. gondii* proliferation. TST and SAHA showed anti-*T. gondii* activity with $IC_{50}$ values of 520nM and 67nM, after 48h treatment, respectively. These IC50 values were used to calculate the selective index with the cytotoxicity assay with MTS. TST and SAHA selectivity index for LLC-$MK_2$ were 38.4 and 88.6, respectively. 25.9 and 68 for i.p macrophages and 38.5 and 29.9 for NDHF and for glial primary culture, respectively. Analysis of the cellular effect of TST and SAHA against *T. gondii* by confocal and super resolution fluorescence microscopy showed that 24-hour treatment affected the parasite individualization process and induced disorganization of cellular structures and organelles such as apicoplast, mitochondrion, centrossome and the internal membrane complex (IMC). These results indicate that TST and SAHA are promising for the treatment of toxoplasmosis as they impair the parasite endodyogeny. **Supported by:**CAPES; CNPQ; FAPERJ **Keywords:** Toxoplasmosis; histone deacetylase inhibitors; chemotherapy

TB28 - **LEISHMANIA AMAZONENSIS INFECTION CONTROL USING METALLOCOMPLEXES AS CHEMOTHERAPY AGENTS: NEW POSSIBILITIES**

LORETE, A.R.M.[*1]; TOSTES, J.M.C.[1]; FERNANDES, C.[2]; JUNIOR, A.H.[2]; [illegible] W.[3]; FORTES, F.S.A.[1]; DAMATTA, R.A.[2]; SEABRA, S.[illegible]
*1.UEZO, RIO DE JANEIRO, RJ, BRASIL; 2.UENF, CAMPOS DOS [illegible]ASIL; 3.UFRJ, RIO DE JANEIRO, RJ, BR*
e-mail:alinelorete@gm[illegible]



Leishmaniasis is a complex disease that is cos[illegible]. The disease is caused by protozoa of the genus Lei[illegible]ors of the subfamily phlebotomine. The species Leishm[illegible]utaneous Leishmaniasis. Therapy for Leishmaniasis ca[illegible]o drug-resistant strains. This situation stimulated the s[illegible] as the use of metallocomplexes. Metallocomplexes ar[illegible] metal core. The action of this compound has been studi[illegible]e family Trypanosomatidae, including species of L. ama[illegible]e effect of compound A3910, which has a cobalt nucle[illegible]s of the WHOM / BR / 75 / Josefa strain. In vitro toxicity [illegible]opy were performed. Preliminary results indicate that A3910 [illegible]orphological alterations of the parasite, such as changes in the shape c[illegible]g of the flagellum, double flagella and invaginations in the parasite's cell bo[illegible]drug was not shown to be toxic to the peritoneal macrophages of mice. Future stu[illegible]nducted to evaluate its mechanism of action. **Supported by:**FAPERJ/CAPES/CNPq **Keyw[illegible]:** Leishmania amazonensis; a3910; metallocomplexes

TB29 - **INTEGRATED TOOL TO ANNOTATE HYPOTHETICAL PROTEINS: A CASE STUDY UTILIZING PROTEOGENOMIC ANALYSIS IN *TRYPANOSOMA RANGELI***

MAIA, G.A.[*1]; SORATTO, T.A.T.[1]; BENETTI FILHO, V.[1]; GRISARD, E.C.[1]; TOLEDO, G.S.[1]; STOCO, P.H.[1]; WAGNER, G.[1]
*1.UNI. FED. SANTA CATARINA, FLORIANOPOLIS, SC, BRASIL.*
e-mail:guiaugmaia@gmail.com

*Trypanosoma rangeli*'s first genome draft presented 66% of the parasite's genes annotated as "hypothetical proteins", which are proteins predicted by bioinformatics software, although their function is unknown. This study aimed to develop a pipeline to attribute annotation to hypothetical proteins through *in silico* analysis based on genomic, transcriptomic and proteomic data of *T. rangeli*. Genes were predicted by Augustus utilizing sequenced and assembled data from different versions of the *T. rangeli* genome. From 10,506 non-redundant proteins, 6,475 were similar to an annotated protein on TriTrypDB (v. 41). Also, 3,740 were similar to annotated hypothetical proteins, 133 as pseudogenes and only 158 did not find any corresponding match, thus resulting in a dataset of 3,898 potential hypothetical proteins. From these, 1,149 had available descriptions or functional annotations considering the results found by InterProScan, HMMER, and RPSblast+, with 788 (20.22%) hypothetical proteins having at least one description. To evaluate the possible expression of these proteins, evidence of expression analysis was performed using available transcriptome and proteomic data from *T. rangeli*. It was found that 3,690 (94.66%) protein sequences had at least one transcript associated and 1,452 (37.25%) at least two different peptides originated from a previous mass spectrometry analysis. Considering only sequences that presented both expression evidence, 1,018 (26.12%) hypothetical proteins could potentially be expressed. Finally, according to the results found here, it is possible to reannotate 372 (9.54%) sequences that were previously annotated as hypothetical, as these are the sequences that show greater evidence. In conclusion, this study developed an integrated systemic analysis that allows for protein reannotation *in silico* and could be applied to other organisms that have available molecular data. **Supported by:**CAPES **Keywords:** Bioinformatics; pipeline; trypanosomatidae

TB30 - **ANTILEISHMANIAL ACTIVITY OF TRIAZOLOQUINOLONES DERIVATIVES.**

SANTOS-PINTO, L.A.[*1]; ANDRADE-NETO, V.V.[1]; RAGUFE, P.[2]; GONÇALVES, A.[2]; SACACINI SAGRILLO, F.[2]; DA COSTA SANTOS BOECHAT, F.[2]; B.V. DE SOUZA, M.C.[2]; DA GAMA OLIVEIRA, V.[2]; CUNHA-JUNIOR, E.F.[1]; TORRES-SANTOS, E.C.[1]

*1.FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.UNIVERSIDADE FEDERAL FLUMINENSE, RIO DE JANEIRO, RJ, BRASIL.*

e-mail:llais.abreu@gmail.com

Studies involving evaluation of structure and activity of synthetic compounds is an approach to discover new drugs and a way to increase the arsenal of treatment of this serious health problem that is the leishmaniasis. Previous work has shown that azols, ergosterol biosynthesis inhibitors and the second-generation quinolones, ciprofloxacin and enoxacin, have potent leishmanicidal activity. Thus, in this work, we planned and synthesized some hybrid derivatives of quinolones and azols, the triazoloquinolones (DQFS series) and their precursors (Quinolone P and QP PROP). The leishmanicidal activity was performed through assays using L. amazonensis promastigotes. The compounds DQFS129, 130 and 131 had an IC50 of 4.0, 7.2 and 6.0 uM, respectively, after 72 h of incubation. The activity was measured using resazurin. The compounds Quinolone P and QP PROP did not show activity against the promastigotes.The toxicity of the compounds was evaluated on peritoneal macrophages, after 72 h of incubation by resazurin method. All DQFS compounds showed CC50 above 50 uM. As follows, the activity of the compounds was evaluated on intracellular amastigotes. Peritoneal murine macrophages were infected with L. amazonensis and treated with the compounds at 10 uM for 72 h. In these conditions, DQFS 129 and 131 reduced the number of amastigotes inside the cells by more than 90%. Altogether, these data point DQFS 129 and 131 as good candidates for in vivo studies. **Supported by:**CNPq e Faperj **Keywords:** Leishmania amazonensis ; drug development; triazoloquinolones

TB31 - **WOULD YOU BE ABLE TO SOLVE ALL THE PUZZLES AND DISCOVER THE CHAGAS' DISEASE IN LESS THAN AN HOUR?**

BARRADAS, M.M.[*1]; DA LUZ, M.R.M.P.[2]; SPIEGEL, C.N.[3]; TORRES-SANTOS, E.C.[2]

*1.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.FIOCRUZ/RJ, RIO DE JANEIRO, RJ, BRASIL; 3.UFF, NITERÓI, RJ, BRASIL.*

e-mail:marcio.mantuano@ioc.fiocruz.br

In a general way, Brazil's major contributions to science are remarkably unknown to the lay public. This lack of scientific culture is critical if we consider mainly the teenagers. In fact, a recent research pointed out that most of them was unable to nominate a single Brazilian scientist. Thus, it is necessary to create mechanisms for improving the scientific communication to assess this age range. Therefore, it is essential to use approaches that arise the interest of the adolescent public. Escape room is a live action game that has been gaining spotlight in Brazil. In an escape room, a group of people cooperate to solve a series of sequential challenges within a defined time, usually an hour. The end of the game often culminates with the final resolution of a central puzzle, which typically results in the door opening, enabling the group to "escape the room". We intend to build scientific-themed escape rooms with themes and puzzles that lead young people to a real immersion in science, making them protagonists of great scientific discoveries, retracing the steps of famous scientists. As the first room, we chose to honor Carlos Chagas, celebrating the 110th anniversary of the discovery of Chagas Disease and what it meant for Science and Public Health. As an example of a puzzle to be solved (spoiler alert) by the players, we introduced a magnetism-activated remote control in the head of a doll (representing Berenice, the first patient diagnosed with Chagas disease). The magnet, that activates the remote control, is hidden under a resin block with a kissing bug inside. The players, noticing certain clues in the room, should approach the kissing bug to Berenice's face, as if discovering how the vector transmits the disease. In doing so, the remote control is activated and a box elsewhere, once locked, is unlocked, releasing another puzzle. The riddles go on and on until the final puzzle, which consists of the discovery of the life cycle of the Trypanosoma cruzi. **Supported by:**FAPERJ **Keywords:** Scientific communication; escape room; games

TB32 - **EPIGALLOCATECHIN-3-GALLATE INHIBIT TRYPANOTHIONE REDUCTASE AS A COMPETITIVE INHIBITOR, LEADING TO A DECREASES IN LEISHMANIA INFANTUM PROMASTIGOTES PROLIFERATION**

FONSECA, M.S.[*1]; INACIO, J.D.F.[1]; CASTRO, H.[2]; TOMÁS, A.[2]; ALMEIDA-AMARAL, E.E.[1]
*1.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.UNIVERSIDADE DO PORTO, PORTO, PORTUGAL.* e-mail:myslene_fsc@hotmail.com

Parasites of the genus Leishmania are obligate intracellular protozoa and survives in a hostile environment caused by the production of reactive oxygen species (ROS) by the host's immune system macrophages. Trypanothione reductase (TR) is an essential enzyme for trypanosomal survival. This enzyme participates in the maintenance of a reducing intracellular environment, protecting parasites from oxidative stress caused by phagocytic cells. Epigallocatechin-3-gallate (EGCG) is a flavonoid that presents leishmanicide effect and capacity to generate ROS. The aim of this study is to demonstrate the effect of EGCG on *L. infantum* promastigote proliferation and the interaction between EGCG and TR. *L. infantum* promastigotes were incubated with EGCG (15.6µM-1000µM) for 72 hours and cell viability was estimated by Alamar blue assay. The EGCG showed a decrease of promastigote proliferation reaching 98% of inhibition at the highest concentration tested (1000µM) and presenting an $IC_{50}$ of 178µM. Molecular docking study showed that EGCG binds at the active site of TR and interacts with residues Thr397, Asn402, His461, Glu466, Ser109' and Try110 with ΔG value of -6.95 kcal/mol. EGCG was capable to decrease the activity of *L. infantum* recombinant TR enzyme in a concentration-dependent manner, presenting a Ki of 199µM. Lineweaver–Burk plot analysis revealed that EGCG was competitive inhibitor corroborating with docking studies. Taken together, our results suggest that EGCG inhibit the *L. infantum* promastigotes proliferation by TR activity inhibition as a competitive inhibitor. **Supported by:**CNPQ, FAPERJ, CAPES, FIOCRUZ **Keywords:** Epigallocatechin-3-gallate; molecular docking; enzymatic kinetics

TB33 - **EVALUATION OF LEISHMANICIDAL ACTIVITY OF EXTRACTS AND FRACTIONS OF HYPNEA SP. ON LEISHMANIA INFANTUM**

BARBOSA, R.C.F.[*1]; OLIVEIRA, M.C.C.[1]; PEREIRA, E.C.[2]; PONTES, M.D.[3]; SOARES, R.O.[3]; TORRES-SANTOS, E.C.[2]; ANDRADE-NETO, V.V.[2]
*1.UFRRJ, RIO DE JANEIRO, RJ, BRASIL; 2.IOC- FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 3.FIPERJ, RIO DE JANEIRO, RJ, BRASIL.*
e-mail:valterfarma@yahoo.com.br

Secondary metabolites involved in the chemical defense of marine organisms need to be potent enough to overcome the diluent capacity of seawater, and thus have the particularity of presenting biological activity even in small doses. This study aimed to test the leishmanicidal activity of extracts and fractions of Hypnea sp., a genus of algae widely distributed along the Brazilian coast, with reports of biological activities, including growth inhibition of Leishmania infantum promastigotes (MINICANTE et al., 2016). The experiments were performed with an initial inoculum of 2.0 x 106 promastigotes/mL of L. infantum and with crude extracts and basic fractions of Hypnea sp., with concentrations ranging from 2 to 128 µg/mL. The parasites were incubated at 26°C for 72 hours. Parasite growth was evaluated by the addition of the resazurin fluorimetric reagent. Acid-base fractionation to obtain basic compounds, such as alkaloids, is justified based on literature data demonstrating the leishmanicidal activity of imidazole-derived alkaloids (ANDRADE-NETO et al, 2011). The crude extract was inactive, while the basic fraction presented IC50 equal to 71.3 µg/mL. Given this result, this fraction was partitioned with methanol and hexane, with the objective of separating the compounds by polarity difference, and the leishmanicidal activity of the obtained subfractions was evaluated. The methanolic subfraction was inactive, while the hexane showed an IC50 of 40.3 µg/mL. This result indicates that, among the compounds present in the basic fraction of Hypnea sp., the substance responsible for bioactivity is among the least polar. Thus, the bioguided fractionation represents an important tool for the isolation of bioactive compounds. The sequence of the work will be through the isolation of the compounds present in the hexane subfraction by classical column chromatography, followed by their characterization by physical analysis methods. **Supported by:**CNPq, CAPES, FAPERJ-RJ. **Keywords:** Benthic algae; leishmanicidal activity; leishmania infantum

TB34 - ***LEISHMANIA* SP. IDENTIFICATION BY HIGH RESOLUTION MELTING ANALYSIS IN CLINICAL CASES OF AMERICAN CUTANEOUS LEISHMANIASIS**

ZAMPIERI, R.A.[*1]; VEASEY, J.V.[2]; LELLIS, R.F.[2]; FREITAS, T.H.P.[2]; FLOETER-WINTER, L.M.[1]
*1.IB-USP, SAO BERNARDO DO CAMPO, SP, BRASIL; 2.HOSPITAL DA SANTA CASA DE SÃO PAULO, SÃO PAULO, SP, BRASIL.*
e-mail:ricardoz@ib.usp.br

Some species from the genus *Leishmania* are agents of American Cutaneous Leishmaniasis (ACL), an infectious dermatosis that affects human under a spectrum of clinical manifestations, dependidg on the species involved in infection and host's immune background. The use of techniques for DNA amplification of parasites based on PCR and the recent application of combined techniques, such as High Resolution Melting (HRM), has been described as a viable alternative for the detection and identification of *Leishmania* sp. in biological samples. The main goal of this work was to identify Leishmania species using a PCR-HRM-based apprach in skin biopsies from hospital-treated patients to establish correlations with histopathological exams, clinical and epidemiological data. A retrospective study was conducted assessing patients with suspected ACL seen at Santa Casa de Misericórdia de São Paulo / Brazil. The paraffin blocks of 22 patients were analyzed by PCR-HRM to confirm the diagnosis and identify the species. From 22 suspected ACL patients, the parasite was identified in 14, comprising five cases (35.6%) of infection by *L. amazonensis*, four (28.5%) by *L. braziliensis*, two (14.4%) by *L. amazonensis* + *L. infantum chagasi,* two (14.4%) by *L. guyanensis* and one (7.1%) by *Leishmania infantum chagasi*. For one of the samples, in which the presence of amastigote forms was confirmed on histopathological exam, the PCR-HRM technique failed to detect the DNA of the parasite. The methodology employed was able to detect and identify *Leishmania* species in paraffin blocks with a sensitivity of 96.4%. The method proved to be useful in clinical samples, thus it can be used as a tool in diagnostic purposes. **Supported by:**FAPESP CNPq **Keywords:** Hrm; diagnosis; paraffin-embedded tissue

TB35 - **STUDY OF THE LEISHMANICIDAL ACTIVITY OF FRACTIONS FROM *AMPELOZIZYPHUS AMAZONICUS***

DE PAULA, L.R.[*1]; MENDONÇA, S.C.[1]; LEITÃO, G.G.[1]; LEITÃO, S.G.[1]; SIMAS, R.C.[1]; SOARES, D.C.[1]; SARAIVA, E.M.[1]
*1.UFRJ, RIO DE JANEIRO, RJ, BRASIL.*
e-mail:leonardordepaula1992@gmail.com

The leishmaniasis is a neglected disease affecting millions of people in 98 countries in tropical and subtropical regions, caused by parasites of the genus Leishmania. This infection can present in many different clinical forms, from the cutaneous form that can heal spontaneously to the visceral form, which can be fatal. The first choice treatment for leishmaniasis are pentavalent antimonials and in the case of failure, amphotericin b or pentamidine, however they have several adverse effects, high costs and also the emergence of resistant parasites has been increasingly reported. In this way, natural products constitute an important source of substances with leishmanicidal potential. *Ampelozizyphus amazonicus* is a plant used in the traditional medicine in the Amazonia for the treatment of malaria . Here, we evaluate the leishmanicidal potential of A. amazonicus against Leishmania amazonensis. Previously we demonstrated that liquid extract from A. amazonicus bark presented low activity against promastigotes with 48% of inhibition of viability after treatment with 800 µg/mL and this same concentration were toxic for 51% of the host cells. Next, we evaluate the leishmanicidal activity from fractions obtained from the etanolic extract of the bark and the wood of this plant. The main fraction of the bark etanolic extract (F3EC), presented IC50 of 81 and 48 µg/mL for promastigotes and amastigotes, while the fraction from F3EC, the F3PA presented IC50 of 20,1 and 7 µg/mL for promastigotes and amastigotes respectively. F3PA presented a superior activity for both parasites forms. On the other hand, the fraction F3EC presented cytotoxicity 10 times smaller than F3PA for the host cells, since F3EC presented CC50 of 91,5 µg/mL and F3PA of 9 µg/mL. Our results suggest that *Ampelozizyphus amazonicus* can be used as a source of substances for the development of leishmanicidal drugs. **Supported by:**CNPq **Keywords:** Leishmanicidal activity ; natural products ; ampelozizyphus amazonicus

TB36 - **EFFICACY OF ITRACONAZOLE-EZETIMIBE-MILTEFOSINE TERNARY THERAPY IN EXPERIMENTAL VISCERAL LEISHMANIASIS**

PISCITELLI, M.C.*[1]; REBELLO, K.M.[1]; PEREIRA, T.M.[1]; TORRES-SANTOS, E.C.[1]; ANDRADE-NETO, V.V.[1]

*1.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL.*

e-mail:matheuscassano.mc@gmail.com

The development of new treatments combining already used with repositioning drugs is an interesting approach for neglected diseases, such as visceral leishmaniasis. Thus, the objective of this work was to evaluate the ternary therapy composed by itraconazole, ezetimibe and miltefosine to the treatment of visceral leishmaniasis. Intracellular amastigotes of Leishmania infantum were incubated with different concentrations of the drugs alone or in combination for 72 hours. None of the combinations was toxic for the macrophages. Itraconazole plus ezetimibe in 3:2 and 1:4 proportions and the ternary combination of itraconazole, ezetimibe and miltefosine in several proportions had synergistic effect. In vivo experiments showed that the ternary drug combination suspended in the ORA-Plus vehicle was the better formulation. The binary combination of ezetimibe and itraconazole and the ternary combination displayed a significant reduction in the parasite load in mice liver and spleen compared to the untreated control and to the treatment with the drugs suspended in milli-Q water. The treatment with the combination of 20 mg/kg plus 200 mg/kg and 3.85 mg/kg of itraconazole, ezetimibe and miltefosine, respectively, decreased in 98% the parasite burden in mice liver and spleen. Altogether, these findings suggest that the use of a suitable vehicle, resulting in a stable formulation, may influence the efficacy of the treatmen. In conclusion, the ternary treatment, composed by miltefosine, itraconazole and ezetimibe, in an adequade vehicle, is a promising therapeutic alternative for the treatment of visceral leishmaniasis. **Supported by:**CNPq, CAPES, FAPERJ-RJ. **Keywords:** Leishmania infantum; azoles; ezetimibe

TB37 - **THE ROLE OF MLPT ON THE *RHODNIUS PROLIXUS* (HEMIPTERA)**

SILVA, C.A.O.*[1]; RODRIGUES, B.C.[1]; ALVES, S.S.[1]; DA SILVA, J.R.[1]; MURY, F.B.[1]; DA FONSECA, R.N.[1]

*1.UFRJ- CAMPUS MACAÉ, MACAÉ, RJ, BRASIL.*

e-mail:carinaa_azevedo@hotmail.com

*Rhodnius prolixus* is a hematophagous insect and vector of *Trypanosoma cruzi*, the causative agent of Chagas disease. A new class of genes encoding small open reading frames (smORFs) was described as essential for several developmental processes. In *R. prolixus* the mlpt plays a role in the regulation of thoracic versus abdominal segmentation, leg development and head formation. However, the most well-studied insect smORF mille-pattes has been thoroughly investigated in holometabolous insects, while its functions in hemimetabolous remain largely unknown. To gain more information on its role we investigated the MLPT function in physiological processes in *R. prolixus*. For this, 63 fifth instar female *R. prolixus* were injected (4µg of dsRNA) into hemocoel with either dsMLPT or dsGFP as control. We dissected the intestinal epithelium of insects at 1, 6, 7, 16 and 19 days after a blood meal (abm) for analysis of gene expression by RT-qPCR. Hemozoin (Hz) was quantified in the midgut 1, 6 and 16 days abm. The protein content was quantified in the anterior midgut (crop) and the native hemoglobin content was accessed in the posterior midgut by native PAGE. Phalloidin staining was performed for morphological analysis. RT-qPCR analysis confirmed a silencing of at least 68%. On days 1 and 6 after a blood meal the Hz content is higher in the dsMLPT in relation to dsGFP. Undigested hemoglobin content was higher at 6, 7 and 19 days abm in dsMLPT injected insects in comparison to dsGFP. The total protein accumulation was more pronounced in the crop of dsMLPT injected insects than in dsGFP. Rpmlpt knockdown caused morphological alterations in anterior midgut and rectum, showing a higher distension that might be due to damages to actin filament. Furthermore, 24 days abm, 88% dsGFP went through molting to adult stage, while those challenged with dsMLPT remained as nymphs. The results suggest possible connections between the MLPT with physiology and molting networks. **Supported by: Keywords:** Smorf mille-pattes; neglected disease; vector

TB38 - **IDENTIFICATION AND CHARACTERIZATION OF HEMOCYTES IN *RHODNIUS PROLIXUS* UPON INFECTION WITH ENTOMOPATHOGENIC FUNGUS *METARHIZIUM ANISOPLIAE***

CUNHA, A.S.S.[*1]; SILVA, C.A.O.[1]; SOUZA, J.F.[1]; BARROS, C.M.[1]; MURY, F.B.[1]
*1.UFRJ-CAMPUS MACAÉ, MACAÉ, RJ, BRASIL.*
e-mail:carinaa_azevedo@hotmail.com

The *Rhodnius prolixus* is a hematophagous insect and vector of *Trypanosoma cruzi*, a protozoan parasite that causes Chagas disease. Insects are exposed to a wide range of microorganisms and have interconnected powerful immune reactions. Innate immunity is the first line of defense being divided into humoral and the cellular response. Hemocytes are cells that circulate in the hemolymph of insects and participate in the cellular immune response. The classification of these cells is based on their morphological differences in overall appearance and nuclear and cytoplasmic characteristics. The *Metarhizium anisopliae* is an entomopathogenic fungus (EF) used as biological control agents and start the infection process mainly by penetration through the insect cuticle. Here we have to classify and analyze morphological changes in hemocytes after *M. anisopliae* infection in *Rhodnius*. The hemolymph of 20 female insects 4 days after rabbit blood feeding, in the presence or absence of EF (1×107 conidia/mL), was collected in 500 µL buffer anticoagulant (0.01 M ethylenediamine tetraacetic acid; 0 0.1 M glucose; 0.062 M sodium chloride; 0.03 M trisodium citrate; 0.026 M citric acid; pH 5.0). Following a time the samples were centrifuged for 10 min at 10,000 xg. In preliminary studies it was possible to observe six morphological hemocyte types were identified by Light Microscopy: Prohemocytes presents high nucleus/cytoplasm ratio. Plasmatocytes are polymorphic cells that can be rounded, oval or ellated, presenting abundant cytoplasm with granules, vacuoles and projections. Oenocytes are rounded, binucleated and eccentric nucleus. Granulocytes have many dense granules and central nuclei. The adipohemocytes are rounded, showing pseudopods and one or more allipidic inclusions. After exposure to EF, a greater recruitment of hemocytes to the site of infection was observed. Thus, information about cell types provides us with a basis for studying the cellular immune system. **Supported by: Keywords:** Hemolymph; hemolymph; immune system

TB39 - **IMMUNE RESPONSE OF *RHODNIUS PROLIXUS* DURING *METARHIZIUM ANISOPLIAE* INFECTION**

ALVES, S.S.[*1]; SILVA, C.A.O.[1]; GUIMARÃES, M.Q.[1]; DE PAULA, A.R.[2]; SAMUELS, R.I.[2]; DA SILVA, J.L.N.[1]; MURY, F.B.[1]; DA SILVA, J.R.[1]
*1.UFRJ - CAMPUS MACAÉ, MACAE, RJ, BRASIL; 2.UENF, CAMPOS DOS GOYTACAZES, RJ, BRASIL.*
e-mail:sandyalves011@gmail.com

The chemical control of triatomines, vectors of Chagas disease, has been threatened in recent years by the appearance of insect populations resistant to pyrethroids. As an alternative approach, the efficacy of the entomopathogenic fungus *Metarhizium anisopliae* was tested against *Rhodnius prolixus* during the late nymphal stage and against adults. However, little is known about the pathogenicity and virulence of *M. anisopliae* against *R. prolixus.* Here we investigated the virulence of this fungus and evaluated the the immune response to fungi infection in nymphs. For the mortality test, nymphs of 1st, 2nd, 3rd, 4th and 5th stages were fed with rabbit blood and then sprayed with fungus ($10^7$ conidia/mL) using Potter tower . In order to analyze the expression of immune genes, Dorsal and Cactus (toll), Relish (IMD), Stat (Jack-Stat) and Defensin were quantified by RT-qPCR 5 days after fungal infection. EF-1 was used as reference. First and second nymph stages present high mortality rates 21 days after fungi infection (88% and 79%, respectively), while 3rd, 4th and 5th stages were more resistant, showing 47%, 43% and 47% mortality respectively. The mortality rates of the controls were around 15%. Analysis of immune genes in the infected insects showed a low expression levels of all genes analyzed in the 1st nymphal stage. Even though 3rd and 4th stages showed a trend toward an increase, the transcripts were still low. Fifth stage were very similar to the controls. The results showed that the 1st and 2snd stages are more susceptible to fungal infection than other stages. It is possible that 1st instar nymphs are unable to mount an efficient immune response to infection. **Keywords:** Neglected disease; biological control; vector

TB40 - **ANTIPROLIFERATIVE EFFECT OF EUSIDERIN A AGAINST LEISHMANIA AMAZONENSIS AND ITS EFFECT ON MITOCHONDRIA FUNCTION**

GOMES, J.S.C.*[1]; FELISBERTO, V.C.[1]; DE PAULA, L.R.[1]; NOGUEIRA DOS SANTOS, C.F [illegible]ES, D.C.[1]; MEYER-FERNANDES, J.R.[1]; SOARES, D.C.[1]; CARBONEZI, L.H.[1]; SARA[illegible]
*1.UFRJ, RIO DE JANEIRO, RJ, BRASIL.*
e-mail:jangomes93@gmail.com



Leishmaniasis is an important public health problem that aff[illegible]orld, and few advances have occurred in relation to its cher[illegible]ill relies on pentavalent antimonials, Amphotericin B and M[illegible]d several side effects leading to patients' withdrawal and to[illegible]cases. All this point heartens the discovery of new drug[illegible]gy, and natural products constitute an important source of[illegible]ial activity.
Piperaceae family contains[illegible]ial activity, such as leishmanicidal, anti-Trypanosoma and[illegible]d the leishmanicidal activity of Eusiderin A, a neolignan is[illegible]abutiloides on Leishmania amazonensis. Our results hav[illegible]of L. amazonensis promastigotes and amastigotes forms[illegible]nt leishmanicidal effect with IC50s of 2.3 µg/mL for pr[illegible] amastigotes and 53 µg/mL for intracellular amastigotes. [illegible]a low toxicity for host macrophages. The anti-leishmanial activity of E[illegible]nt of reactive oxygen species (ROS), nitric oxide (NO) and TNF-α pr[illegible]ages, and it did not affect arginase activity in these cells. Eusiderin A did not affe[illegible] plasma membrane permeability assayed by Sytox green or induce the exposure of ar[illegible]ligands, as well. Our data have shown that the mitochondrial membrane potential and oxyge[illegible]low of promastigotes are affected by Eusiderin A, which also induces accumulation of neutral lipid bodies, suggesting that this neolignan causes incidental death of the parasites. **Supported by:**Capes, CNPq, FAPERJ **Keywords:** Leishmania amazonensis; eusiderin a; antileishmanial activity