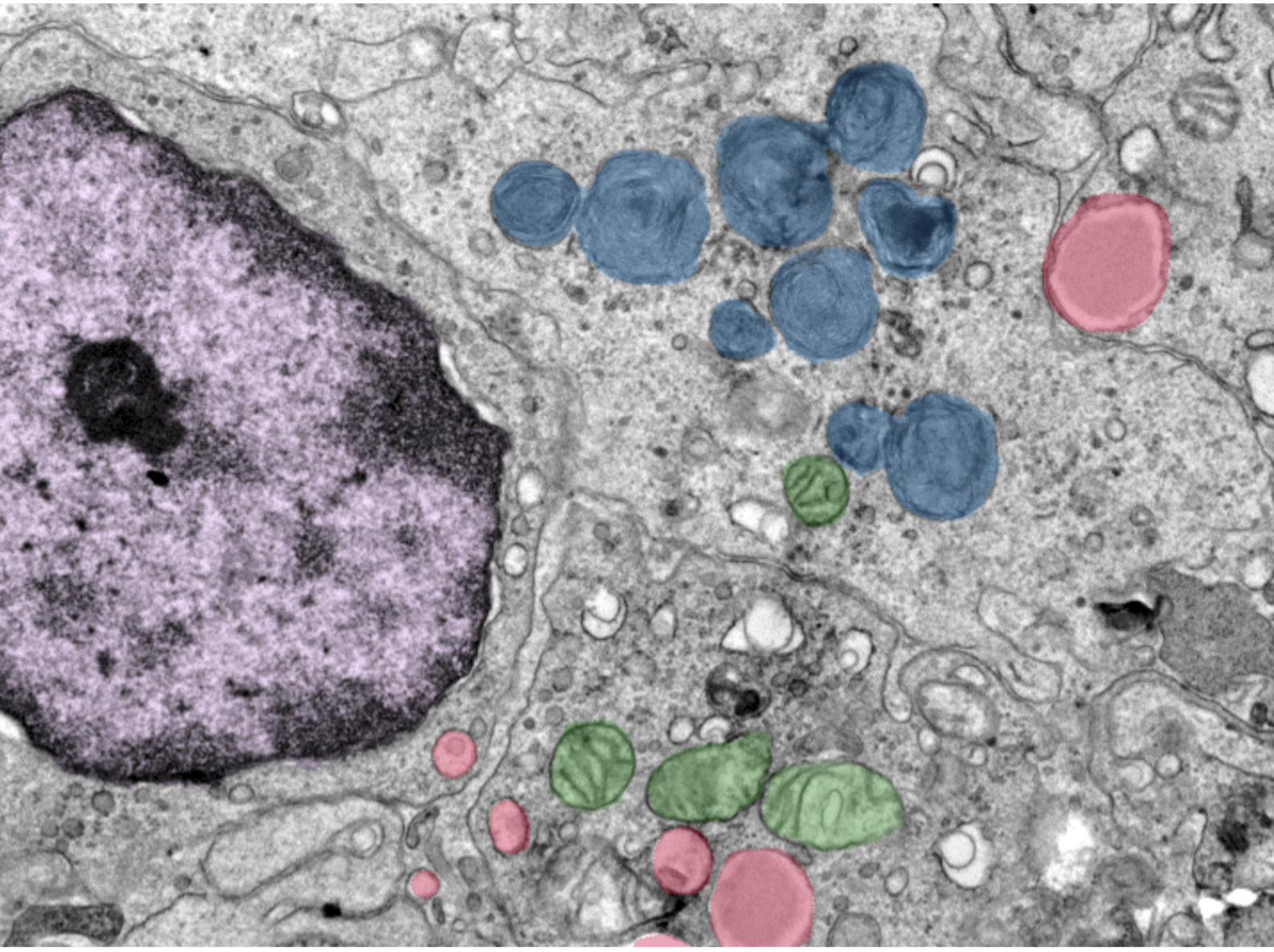
XXXVIII
Annual Meeting of the Brazilian Society of Protozoology

XLIX Annual Meeting of Basic Research in Chagas' Disease



Sept 04 - 06, 2023
Hotel Gloria | Caxambu, MG

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www.sbpz.org.br

PROCEEDINGS

XXXVIII Meeting of the Brazilian Society of Protozoology
XLIX Annual Meeting on Basic Research in Chagas' Disease

*Hotel Glória, Caxambu, MG, BRASIL- C*axambu
4-6 September, 2023

Colegiado Diretor SBPz

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Dear Participants,

On behalf of the Brazilian Society of Protozoology (SBPz) and the Organizing Committee, it is a great pleasure to welcome you to the **XXXVIII Meeting of the Brazilian Society of Protozoology** and the **XLIX Annual Meeting of Basic Research in Chagas´ Disease**.

Our goal to this year's meeting was to put together a scientific program as broad as possible so that we could bring new colleagues to our meeting. As usual, we had numerous suggestions to the program some of which we were not able to include in the final program due to time and space limitations.

As usual we invited speakers from many countries, especially from South America, and from different regions of Brazil in an attempt to bring to you the best science in the field of Protozoology. A total of nine conferences and six round tables with four speakers in each will discuss the advances in the area. In addition, two sessions of oral presentations and two poster sessions will allow for students and post-docs to present and discuss their work. The Walter Coli and Zigman Brener Awards will be given to the best presentations and poster in each category.

This year's Samuel Pessoa Award will be granted to Professor Edgar de Carvalho, from UFBA, in recognition to his outstanding contribution to the field of leishmaniasis.

Continuing with our initiative to encourage Early Career Researchers to participate in our society, one of the round tables was organized by a committee of young scientists who invited four colleagues to present their work. We encourage all of you to attend to the round table.

We would like to acknowledge the invited speakers, SBPz members, colleagues, and students for supporting and attending this meeting. As always, we are particularly indebted to the SBPz Secretariat, Mrs Ana Paula Lopes Vidal and Mrs Vilma de Araújo Andrade, whose commitment and dedication over the years, along with the Board of Directors, have assured the persistence of the meeting, even in difficult times like the one Brazilian Science was going through recently, guaranteeing the quality and international recognition of the meeting. We would also like to acknowledge the financial support received from FAPESP, FIOCRUZ, ISOP and The Company of Biologists (UK).

We welcome you all to our meeting, hoping that the scientific program covers expectations and stimulates the high-level discussions that have always characterized our meetings

São Paulo, September 4th, 2023

Luís Carlos Crocco Afonso
President of SBPz

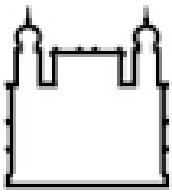
FOTO DA CAPA/COVER BOOK

Legenda da Figura

A - Photomicrograph of transmission electron microscopy of adipose tissue in obese cutaneous leishmaniasis.
A1 - Lipid bodies phagocytosed by macrophages.
A2 - Morphological changes of mitochondrias.

Dr. Edgar de Carvalho

FINANCIAL SUPPORT



FIOCRUZ
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SEPTEMBER, 4th, 2023

16h30 – 17h00 | **OPENING CEREMONY**
Room A

17h00 – 18h00 | **OPENING CONFERENCE** **ROOM A**
SAMUEL PESSOA AWARD
CHAIR: Camila Indiani de Oliveira

Control and Pathology in *Leishmania braziliensis* Infection: Lessons from Corte de Pedra
Edgar Marcelino de Carvalho *(Fiocruz – BA – Brazil)*

18h00 – 19h30 | **BRAINSTORM** **Room A**
Chair: Jennifer Ann Black

Preprint publishing in Science

19h00 – 20h30 | **DINNER**

20h30 – 22h30 | **POSTER SESSION – ODD** **GYMNASIUM**

09h00 – 11h00 | **Oral Presentations** **ROOM A**
OP.01

Chairs: Renata Tonelli and Walter Colli

HP01 – Iron and heme status at the *Leishmania*-host interface: effect of iron deficiency anemia on the *Leishmania (L.) amazonensis* virulence.
AHYUN AVERY HONG

HP02 – Functional characterization of the Asparagine Synthetases of *Trypanosoma cruzi*
ANDREA KATHERINE PINTO MARTINEZ

HP03 – First report of *Leishmania* RNA virus 2 (LRV2) in *Leishmania infantum* strains from canine and human visceral leishmaniasis in the Southeast of Brazil
RODRIGO PEDRO PINTO SOARES

HP04 – Phase I and II of NasoLeish®, a novel vaccine against canine visceral leishmaniasis caused by *Leishmania infantum*.
OTONI ALVES DE OLIVEIRA MELO JUNIOR

TB01 – Rna Binding Proteins As Trans-Regulators Impacting Surveillance And Infectivity In *Leishmania*.
NATÁLIA MELQUIE MONTEIRO-TELES

TB02 – Development Of Amphotericin B Microparticulated Implants By Spray-Drying For Local Treatment Of Cutaneous Leishmaniasis
FELIPE CARVALHO GONDIM

TB03 – A genome-wide yeast surface display screen to identify vaccine targets for Chagas disease
MIRA LOOCK

TB04 – Whole genome analysis of paromomycin resistant lines and susceptible isolates of *Leishmania amazonensis* reveals changes in chromosomal somy and variable polymorphisms
ELIZABETH MAGIOLO COSER

TB05 – Paving the road for genomic surveillance of leishmaniasis: source tracing of *L. donovani* in recent outbreaks of visceral leishmaniasis in West Nepal
JEAN-CLAUDE DUJARDIN

TB06 – Antileishmanial of Drugs Identified by High Content Screening against Intracellular Amastigotes
LETÍCIA DE ALMEIDA

TB07 – Search for new Drugs and Potential Molecular Targets Associated with Benznidazole Resistance Phenotype in *Trypanosoma cruzi*
DAVI ALVARENGA LIMA

09h00 – 11h00 | **Oral Presentations** **ROOM B**
OP.02

Chairs: Angela Hampshire and Ana Maria Murta

PV01 – Role of ?-tubulin acetylation in the cell cycle of *Trypanosoma cruzi*
VICTORIA LUCIA ALONSO

PV02 – Development of paratransgenic Lutzomyia longipalpis by engineered bacteria driving refractoriness to *Leishmania infantum chagasi* infection.
ANA CAROLINA PEDRO SANTOS RIBEIRO

PV03 – The three-dimensional genome organization in *Trypanosoma cruzi*
NATÁLIA KARLA BELLINI

PV04 – Iron Uptake Controls *Trypanosoma cruzi* Metabolic Shift and Cell Proliferation
CLAUDIA FERNANDA DICK

PV05 – Mitochondrial inheritance in *Toxoplasma gondii* is dependent on actin and myosin A
RODOLPHO ORNITZ OLIVEIRA SOUZA

PV06 – Accessing The Genomic Elements And Structural Variants Between *Crithidia Sp* LVH60a AND *C. Fasciculata* Genomes
LUANA APARECIDA ROGERIO

PV07 – Loss of RNase H1 in Leishmania leads to an altered DNA replication programme and increased genome variability.
JEZIEL DENER DAMASCENO

PV08 – Diversification of Heterotrophic Microeukaryotes: Insights from Arcellinida and Related Amoebozoan Taxa
DANIEL JOSÉ GALAFASSE LAHR

PV09 – Unveiling a novel Isoprenoid Salvage Pathway in *Plasmodium falciparum*: new perspectives in fosmidomycin as an antimalarial
MARCELL CRISPIM

PV10 – Molecular Insights into the Evolutionary Origin and Mechanisms of Shell Formation in Amoebozoan Testate Amoebae (Arcellinida:Amoebozoa)
ALFREDO LEONARDO PORFÍRIO DE SOUSA

PV11 – Emerging-3'UTR ncRNAs of duplicated genes in Leishmania major: what is their relevance?
JOSÉ CARLOS QUILLES JUNIOR

PV12 – Recombinant Expression of *Trypanosoma cruzi* Histones for Nucleosomes Assembly
MATHEUS ISSA

11h00 – 11h30 | **Coffee break**

11h30 – 12h10 | **CONFERENCE 01** **ROOM A**
Chair: Didier Jean Jacques Salmon

Cyclic AMP signaling and evolution of unusual protein kinase A in kinetoplastids
Michael Boshart *(Universität München – Germany)*

12h30 – 14h30 | **Lunch**

13h30 – 14h30 | **Council Meeting**

14h30 – 16h30 | **Round-Table** **ROOM A**
RT.01 – Immunity against protozoans
Chair: Tiago Wilson Patriarca Mineo

Exhaustion on *L. amazonensis* and *L. brasiliensis* infection
Herbert Guedes *(UFRJ – Brazil)*

Heme Trafficking at the Leishmania-Host Interface
Iqbal Hamza *(University of Maryland – USA)*

Regulation of Immunological Memory to *Toxoplasma gondii*
Kirk Jensen *(University of California – USA)*

The immune response to *Trypanosoma cruzi* infection is driven by the purinergic system
Maria Del Pilar Aoki *(Universidad Nacional de Córdoba – Argentina)*

14h30 – 16h30 | **Round-Table** **ROOM B**
RT.02 – Protozoan Vectors and Viruses
Chair: Yara Maria Traub-Cseko

Viruses and antiviral defenses in Rhodnius prolixus
Attilio Pane *(UFRJ – Brazil)*

Identification of Endogenous Viral Elements (EVEs) in different populations of Sandflies in Brazil
Antonio Tempone *(FIOCRUZ – RJ – Brazil)*

Insights into the Interaction between protozoan parasite vectors and Viruses
Eric Aguiar *(UESC – Brazil)*

16h30 – 17h00 | **Coffee break**

17h00 – 17h30 | **Conference 02** **ROOM A**
Chair: Luis Carlos Crocco Afonso

Immune Responses In Naive Dogs Exposed To Uninfected *Lutzomyia Longipalpis* Bites
Claudia Brodskyn *(FIOCRUZ-CPqGM – BA – Brazil)*

17h00 – 17h30 | **Conference 03** **ROOM B**
Chair: Narcisa Leal da Cunha-e-Silva

Trichomonas vaginalis extracellular vesicles in cell communication
Natalia de Miguel *(INTECH – Argentina)*

17h30 – 18h00 | **Conference 04** **ROOM A**
Chair: Jeffrey Shaw

Exploring host-specificity: Unraveling the relationship between *Leishmania*
Elisa Cupolillo *(FIOCRUZ – RJ – Brazil)*

17h30 – 18h00 | **Conference 05** **ROOM B**
Chair: Erica Duarte

The ultrastructure of cell division coordination in *Toxoplasma gondii*
Maria Eugênia Frância *(Institut Pasteur de Montevideo − Uruguay)*

18h30 – 19h30 | **Plenary SBPz – Ordinary General Assembly** **ROOM B**

19h30 – 20h30 | **Dinner**

20h30 – 22h30 | **Poster Session – EVEN** **GYMNASIUM**

09h00 – 11h00 | **Round-Table** **ROOM A**
RT.03 – Fascinating peculiarities regarding protozoan parasites
Chair: MARCELO S. DA SILVA

Uncovering the Mechanisms of VSG mRNA Regulation through Multi-Omics Analysis
Esteban Erben *(UGSAM – Argentina)*

The chromatin and protein acetylation in *Toxoplasma gondii*
Sheila Nardelli *(FIOCRUZ – PR – Brazil)*

Synthesis and translation of selenocysteine in Kinetoplastida and Bacteria
Otávio H. Thiemann *(USP – Brazil)*

Total parasite biomass but not peripheral parasitaemia is associated with endothelial and haematological perturbations in *Plasmodium vivax* patients
Fábio T. M. Costa *(UNICAMP – Brazil)*

09h00 – 11h00 | **Round-Table** **ROOM B**
RT.04 – Current Topics in Protozoology
(organized by the Early Career Research (ECR) committee)
Chair: Maria Fernanda Laranjeira-Silva

Modeling of epidemiological parameters in vector-borne diseases
Gabriel Zorello Laporta *(Fundação ABC – Brazil)*

Integrative systems biology of host-Plasmodium interactions
Luiz Gustavo Araujo Gardinassi *(UFG – Brazil)*

Unraveling the mechanisms of endocytosis by intracellular amastigotes of *Trypanosoma cruzi*
Carolina Alcantara *(UFRJ – Brazil)*

What are trypanosomatids presumably exclusive to insects doing in human hosts? A closer look at isolation of *Crithidia* parasites from visceral leishmaniasis patients in Sergipe, Brazil.
Sandra Maruyama (UFSCAR – Brazil)

11h00 – 11h30 | **Coffee break**

11h30 – 12h00 | **Conference 06** **ROOM A**
Chair: Renata Rosito Tonelli

Implications of *G. lamblia* extracellular vesicle functions for inter-pathogen communication
Maria Carolina Touz *(UNICET – Argentina)*

12h00 – 12h30 | **Conferences 07** **ROOM A**
Chair: Daniel José Galafasse Lahr

Characterizing the diversity of eukaryotes in the primate gut
Laura Parfrey *(UBC – Canada)*

12h30 – 14h30 | **Lunch**

14h30 – 16h30 | **Round-Table** **ROOM A**
RT.05 – Progress in Vaccine Development and Diagnostic tests for parasitic diseases
Chair: Santuza M. Ribeiro Teixeira

Toxoplasma gondii vaccines: The past, present, and future
João Luis Garcia *(UEL – Brazil)*

Non-clinical toxicity and immunogenicity evaluation of repeated administration of a *Plasmodium vivax* malaria vaccine in mice and rabbits
Irene Soares *(USP – Brazil)*

Exploring miRNAs as potential biomarkers using an experimental model of chronic Chagas disease cardiomyopathy
Otacílio Moreira *(FIOCRUZ – RJ – Brazil)*

LeishID: LAMP-based molecular diagnostic tool for species-specific Leishmania detection
Rubens do Monte *(FIOCRUZ – MG – Brazil)*

14h30 – 16h30 | **Round-Table** **ROOM B**
RT.06 – Genomics
Chair:João Marcelo Pereira Alves

Genomes of Endotrypanum schaudinni and Zelonia costaricensis: Expansion of multigene families in Leishmaniinae parasites that are close relatives of *Leishmania spp.*
João Marcelo Pereira Alves *(USP − Brazil)*

VEuPathDB: Omics support for the global parasite, vector and fungal research communities
Omar Harb *(University of Pennsylvania − USA)*

Mosaic aneuploidy in *Leishmania* and its role in adaptation.
Malgorzata Domagalska *(University of Antwerp − Belgica)*

Updated Genome Assemblies and Metagenomics of Phlebotomus papatasi and Lutzomyia longipalpis
Mary Ann McDowell *(University of Notre Dame − USA)*

16h30 – 17h00 | **Coffee-Break**

17h00 – 17h30 | **Conference 08** **ROOM A**
Chair: Rodrigo Pedro Pinto Soares

RNA editing in non-model kinetoplastids
Vyacheslav Yurchenko *(University of Ostrava – CZ)*

17h00 – 17h30 | **Conference 09** **ROOM B**
Chair: Julio Scharstein

Update on Malaria-Associated Acute Respiratory Distress Syndrome
Sabrina Epiphanio *(USP – SP – Brazil)*

17h30 – 18h30 | **Closing Conference** **ROOM A**
Chair: Elvira Maria Saraiva

What we have learned about *Trypanosoma cruzi* endocytic pathway
Narcisa Leal da Cunha-e-Silva *(UFRJ – RJ-Brazil)*

18h30 – 19h30 | **Closing Ceremony, Remarks and Awards** **ROOM A**

19h30 – 21h30 | **Dinner**

SPC-1 – **Opening Conference – Samuel Pessoa Award**
Control and Pathology in *Leishmania BRAiensis* Infection: Lessons from Corte de Pedra

DE CARVALHO, A.M.P.[1]; BACELLAR, O.A.[2]; DE OLIVEIRA, C.I.[1]; DE CARVALHO, L.P.[1]; OLIVEIRA, S.C.[3]; ARRUDA, S.[4]; DE CARVALHO FILHO, E.M.[1].
1. FEDERAL UNIVERSITY OF BAHIA, BRA; 2. IMUNOLOGY SERVICE, HOSPITAL UNIVERSITÁRIO PROFESSOR EDGARD SANTOS, BA - BRA; 3. INSTITUTE OF BIOMEDICAL SCIENCES, DEPARTMENT OF IMMUNOLOGY, UNIVERSITY OF SÃO PAULO, SP - BRA; 4. GONÇALO MONIZ INSTITUTE (IGM), FIOCRUZ, BA - BRA.
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A Southeastern region of the state of Bahia, BRA, is one of the most important endemic areas of American tegumentary leishmaniasis (ATL). Studies in this place started in the village of Três Braços, but after an outbreak of ATL in Corte de Pedra in 1984 a Reference Center for Diagnosis and Treatment of ATL was built in 1987. Initially, both Leishmania (Viannia) BRAiensis and Leishmania (Mexicana) amazonensis were isolated, but in the last 25 years only *L. BRAiensis* is the causal agent of the disease. Cutaneous leishmaniasis (CL), disseminated leishmaniasis (DL) and mucosal leishmaniasis (ML) are documented in about 90%, 6% and 3% respectively in this area. Moreover, about 22% of household contacts of CL patients are infected with *L. BRAiensis* but do not develop disease and are considered as having subclinical *L. BRAiensis* infection (SC). The control of leishmania is mainly mediated by macrophage (Mφ) activation by IFN-γ but in CL, ML and DL despite a strong Th1 immune response enough parasite persists. In contrast a weak Th1 immune response is observed in SC. Mφ from SC have a low respiratory burst but kill more efficiently leishmania than Mφ from CL. While NO is produced in low levels in human Mφ infected with *L. BRAiensis*, ROS participate in the control of parasite growth. Inhibition of ROS increase parasite load in Mφ from CL, ML and DL, but in subjects with SC infection inhibition of ROS and NO does not change the parasite load. In these individuals leishmania killing occurs rapidly after infection and is associated with a quick maturation of the phagolysosomes. Mφ of subjects with SC are also less susceptible to parasite internalization. CL is characterized by one or more round shape well limited ulcer with raised borders. ML is mainly observed in the nose and appears years after a primary CL but in 17% of the cases the mucosal involvement is concomitant with the cutaneous ulcer. DL is characterized by more than 10 and up to 1000 papular and ulcerated lesions in different areas of the body. Both parasite and host factors participate in the pathogenesis of CL. *L. BRAiensis* in the region of Corte de Pedra is polymorphic and genotypic differences in haplotypes of the chromosome 28 among isolates are associated with severity of the disease and failure to therapy. For instance, isolates of DL are more internalized than isolates of CL in Mφ with both forms of the disease, but isolates of DL proliferate more in DL Mφ than in cells from CL patients. The pathology in ATL is associated with an exaggerated Th1 immune response with high production of IFN-γ, TNF, IL-1β, IL-6 and Granzyme B. Parasites are scarce in histopathologic analysis but parasite load detected by DNA of *L. BRAiensis* or by labelled anti *L. BRAiensis* antibody is high. Both the inflammatory infiltrate and parasite load are associated with severity of the disease and failure to therapy. Activated CD8+ T cells and NK cells are present in the inflammatory infiltrate and together with IL-1β and inflammasome play a key role in the killing of cells and development of the ulcer. A high number of B cells and plasma cells are documented at the lesion site and regulatory B cells produce IL-10 which may contribute to parasite persistence in Mφ. Dermal M1 and M2 Mφ are infected with *L. BRAiensis* but while the majority of infected Mφ have only one or few amastigotes a small number of cells have high parasite load suggesting that Mφ subsets remain infected with a large number of amastigotes. Treatment for CL is a challenge in Corte de Pedra as failure to meglumine antimoniate (MA) is observed in up to 50% of the cases. Additionally, those who respond to therapy take 60 to 90 days to achieve cure. As pathology in ATL is associated with the host inflammatory response, the combination of MA with immunomodulatory drugs increase the cure rate and decrease the healing time of cutaneous ulcers.
Keywords: Cutaneous leishmaniasis;L; BRAiensis;Pathogenesis; Therapy.

CO-01 - **Cyclic AMP signaling and evolution of unusual protein kinase A in kinetoplastids**

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Stage development and host adaptation of vector transmitted parasites require fast and reliable perception of signals from the host environments. Kinetoplastids share with model eukaryotes second messengers and signaling modules like protein kinases, but their connections, activation mechanisms and interaction in signaling pathways differ mostly from the well investigated models. The second messenger cAMP is important for innate immunity subversion of trypanosomes in the mammalian host[1] and for transmission via colonization of the tsetse salivary glands[2]. We begin to understand the role of the large adenylate cyclase family and a new multi-cyclase regulator (CARP3) in these processes. The cAMP effector protein(s) in this pathway are still elusive. Notably, protein kinase A (PKA), the primary mammalian cAMP effector, has lost its regulation by cAMP. Nucleoside ligands adopted this role[3]. The structural basis and evolutionary origin of ligand and activation specificity of PKA orthologues and paralogues has been investigated by crystallography, binding assays and a large number of site directed mutants in vitro and in vivo. The repurposing of PKA for novel ligands and pathways other than cAMP evolved in the Euglenozoa. In *T. brucei*, PKA is activated by two environmental cues that trigger differentiation: low temperature (cold shock) and carbon source availability. In *Leishmania*, another unusual PKA isoform tehered to the subpellicular microtubules is essential for maintenance of the elongated shape of promastigotes. Multiple roles in essential adaptive processes in the parasitic life cycle and the unique ligand specificity thus suggest PKA as an attractive target to therapeutically address parasitic diseases caused by kinetoplastids.

1. Salmon, D., Vanwalleghem, G., Morias, Y., Denoeud, J., Krumbholz, C., Lhomme, F., Bachmaier, S., Kador, M., Gossmann, J., Dias, F.B.S., et al. (2012). Adenylate Cyclases of Trypanosoma brucei Inhibit the Innate Immune Response of the Host. **Science** 337, 463-466. 10.1126/science.1222753.
2. Bachmaier, S., Giacomelli, G., Calvo-Alvarez, E., Vieira, L.R., Van Den Abbeele, J., Aristodemou, A., Lorentzen, E., Gould, M.K., Brennand, A. et al. (2022). A multi-adenylate cyclase regulator at the flagellar tip controls African trypanosome transmission. **Nat Commun** 13, 5445. 10.1038/s41467-022-33108-z.
3. Bachmaier, S., Volpato Santos, Y., Kramer, S., Githure, G.B., Klockner, T., Pepperl, J., Baums, C., Schenk, R., Schwede, F., Genieser, H.G., et al. (2019). Nucleoside analogue activators of cyclic AMP-independent protein kinase A of Trypanosoma. **Nat Commun** 10, 1421. 10.1038/s41467-019-09338-z.
contact presenting author at boshart@lmu.de **Keywords**: signaling;cyclic AMP;protein kinase A.

CO-02 - **IMMUNE RESPONSES IN NAIVE DOGS EXPOSED TO UNINFECTED *LUTZOMYIA LONGIPALPIS* BITES**

BRODSKYN, C.[1]; SOLCÀ, M.D.S.[2]; SANTOS, Y.[2]; JESUS, S.C.S.[3]; COELHO, A.M.R.M.[2]; MACEDO, B.[1]; KAMHAWI, S.[4]; VALENZUELA, J.G.[4]; FRAGA, D.B.M.[3].
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Canine visceral leishmaniasis (CVL) is a disease caused by the parasite Leishmania infantum and transmitted to both dogs and humans through the bites of infected sandflies. In BRA, the primary vector responsible for transmitting the disease is the sandfly species Lutzomyia longipalpis. During feeding, infected sandflies inject metacyclic promastigote forms of the Leishmania parasite, along with their saliva and other components, into the host's bloodstream. Research has shown that antibodies against sandfly saliva are associated with increased severity of visceral leishmaniasis in naturally infected dogs. At the bite site, different inflammatory responses occur, which are mediated by salivary proteins, exosomes and promastigote secretory gel, as well as the microbiota of the sandfly. All of these compounds modulate the host´s hemostatic, inflammatory and immune responses, directly influencing the establishment of Leishmania infection. The early inflammatory response of naïve dogs locally at the site of the sandfly bite can identify which components are more

likely to influence the successful establishment of Leishmania infection In order to assess early skin immune response to uninfected sandflies bites, six dogs were employed: Three 3mm diameter skin biopsies were collected before the exposure from each dog. Exposure was performed using uninfected sandflies from the IGM colony. Total RNA was extracted and the integrity and quantification of the mRNA was confirmed by automated electrophoresis and the cDNA library was built using the Ion total RNA-seq V2 kit. The majority, approximately 70%, of the 295 DEGs (differentially expressed genes) identified by the LinDA analysis were related to the first time point evaluated, 4 hours after saliva exposure. The 24-hour time point had fewer DEGs, while a larger number were present at the later evaluated time point, 48 hours. About 13% of the DEGs could not be mapped in the enrichment analysis. Thus, this analysis was performed using the remaining 255 DEGs. These genes revealed 28 enriched pathways after exposure to sand fly saliva. The IL-17 signaling pathway was the only pathway related to the 24-hour time point and was also associated with the initial 4-hour time point. Pathways related to leishmaniasis, tuberculosis, phagosomes, and chemokine signaling were enriched only at the later evaluated time point. Out of the 28 enriched pathways, 18 were specifically associated with the initial time point. These pathways were associated with the inflammatory response and some commonly associated with chronic diseases such as Chagas disease, African trypanosomiasis, and rheumatoid arthritis. Cytokine genes related to IL1B, IL6, CXCL8, and CCL2 were shared by different pathways associated with the 4-hour time point and some pathways related to the 48-hour time point. Since the FCGR1A gene is connecting pathways related to the early time point after exposure to sand fly saliva with those related to the later 48-hour time point, it may be playing a role in the response at these two time points. Additionally, antibodies against sandfly saliva can be useful in assessing vector exposure in endemic areas. Recombinant proteins derived from sandfly saliva, such as LJM11 and LJM17, have been employed to detect anti-saliva antibodies in dogs. In an experimental study, six dogs were exposed to Lu. longipalpis sandflies, and their production of anti-LJM11 and anti-LJM17 antibodies was monitored over time. The dogs showed an immediate increase in antibody production after the first exposures, and the antibody titers remained detectable for over a year, with variations observed among individual animals. Upon re-exposure to sandflies, the dogs exhibited a significant rise in antibody titers. This study allows us to better understand of immune responses in dogs, contributing to the design of new prophylactical vaccines, considering the role played by the sand flies in the establishment of infection.
Supported by: PROEP IGM-FIOCRUZ N°01/2020 and Fulbright Junior Member Faculty Award
Keywords:: Sandfly;Saliva;Antibodies; Reservoir.

CO-03 - **Extracellular vesicles from the parasite *Trichomonas vaginalis:* role in cell communication**

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Trichomonas vaginalis is a common sexually transmitted parasite that colonizes the human urogenital tract where it remains extracellular and adheres to epithelial cells. Infections range from asymptomatic to highly inflammatory, depending on the host and the parasite strain. With an estimated annual prevalence of 276 million new cases, mixed infections with different parasite strains are expected. Although it is known that parasites interact with their host to enhance their own survival and transmission, evidence of mixed infections call into question the extent to which unicellular parasites communicate with each other. We recently demonstrated that different T. vaginalis strains can communicate through the formation of cytoneme-like membranous cell connections. We showed that cytonemes formation of an adherent parasite strain (CDC1132) is affected in the presence of a different strain (G3 or B7RC2). Our findings provide evidence that this effect is contact-independent and that extracellular vesicles (EVs) are responsible, at least in part, of the communication among strains. EVs are heterogeneous membrane vesicles released from virtually all cell types that collectively represent a new dimension of intercellular communication. We found that EVs isolated from G3, B7RC2, and CDC1132 strains contain a highly distinct repertoire of proteins, some of them involved in signaling and communication, among other functions. Finally, we showed that parasite

adherence to host cells is affected by communication between strains as binding of adherent *T. vaginalis* CDC1132 strain to prostate cells is significantly higher in the presence of G3 or B7RC2 strains. We also observed that a poorly adherent parasite strain (G3) adheres more strongly to prostate cells in the presence of an adherent strain. The study of signaling, sensing, and cell communication in parasitic organisms will enhance our understanding of the basic biological characteristics of parasites, which may have important consequences in pathogenesis.
Supported by:NIH **Keywords:** parasite;communication;vesicles.

CO-04 - **Exploring host-specificity: Unraveling the relationship between *Leishmania (Viannia)* species and its endosymbiont *Leishmania* RNA Virus 1**

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An important aspect in the epidemiology of Tegumentary Leishmaniasis (TL) in endemic regions of northern South America, as well as certain areas in Central America, is the presence of Leishmania parasites carrying a viral endosymbiont known as Leishmania RNA Virus 1 (LRV1). LRV1 is a double-stranded RNA virus found in various species of Leishmania (Viannia). Some studies have indicated that Leishmania parasites carrying LRV1 are more likely to cause severe symptoms of TL, increasing the risk of progression to the mucosal form of the disease. However, while the association between LRV1 and mucosal manifestation has not been observed in all studies, there is evidence linking LRV1 to therapeutic failure. These inconsistencies may be attributed to differences in the parasite populations circulating in the various regions studied and/or variations in the LRV1 strains infecting these parasites. LRV1 has been detected in cultivated strains of *L. BRAiensis*, *L. guyanensis*, *L. naiffi*, *L. panamensis*, and *L. shawi*, but not in other *Leishmania (Viannia)* species. Although LRV1 has been associated with human infections caused by *L. lainsoni* and *L. peruviana*, cultivated strains of these species have yet to be identified as LRV1 positive. Previous studies employing complete genome sequences of LRV1 and sequences obtained from a phylogenetically informative region of the viral genome from *L. guyanensis* and *L. BRAiensis* have demonstrated evidence of host-specificity in the interaction between *L. (Viannia)* species and LRV1, with LRV1 sequences clustering according to their respective Leishmania species hosts. Host-specificity has also been observed in the analysis of LRV1 from *L. shawi* and *L. naiffi*. LRV1 from L. shawi clustered closely with LRV1 from L. guyanensis, mirroring the observed phylogenetic relationship between these two Leishmania species. All LRV1 sequences from *L. naiffi* clustered together, forming the most divergent group. It is worth noting that phylogenetic analysis suggests *L. naiffi*, along with *L. lainsoni*, as the most divergent species within theViannia subgenus. Given the significance of the Leishmania-LRV1 symbiosis in the epidemiology of cutaneous and mucosal leishmaniasis, it is crucial to obtain a comprehensive understanding of the diversity and spread of this virus within parasite populations.
Keywords:: Leishmania (Viannia);Leishmania RNA Virus 1;host-specificity.

CO-05 - **The ultrastructure of cell division coordination in *Toxoplasma gondii***

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The ability of protozoan parasites to rapidly proliferate within their host is at the core of their mechanisms of pathogenesis. Obligate intracellular parasites of the apicomplexan phylum lyse their host cells and tissues as a consequence of their cell division. Parasites of this phylum resort to flexible cell division modes resulting in variable outputs. *Toxoplasma gondii*, the causative agent of toxoplasmosis, for example, is able to proliferate by means of endodyogeny, endopolygeny and schizogony. These modes of division share mechanistic features such as lack of chromatin

condensation, nuclear fission by semi-closed mitoses and *de novo* daughter cell assembly. However, the underlying mechanisms of this flexibility have only recently started to emerge. The centrosome, one of the microtubule organizing centers in the cell, has long been staged at the center of regulation. Here, we have dissected the contribution of different centrosomal components in *T. gondii*, highlighting their individual contributions orchestrating distinct phases of endodyogeny. Using ultrastructure expansion microscopy, we have analyzed the phenotypes displayed by a number of conditional mutants of centrosomal proteins uncovering their unexpected roles in microtubule nucleation, centriole biogenesis and regulation. Overall, our work proposes a model for the modular organization of centrosomal functions which ultimately underlies cell division flexibility, allowing these parasites to adapt to different niches and proliferate accordingly. Su**pported by:** Pasteur Network
Keywords:: Toxoplasma gondii;Cell Division;UExM.

CO-06 - **Exploring Exosome-Like Vesicles in *Giardia lamblia*: Implications for Parasite Communication, Pathogenicity, and Drug Resistance**

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Extracellular vesicles (EVs) play a vital role in intercellular communication and their potential to promote pathogenesis in parasites has generated significant interest in the medical field. These vesicles involve microvesicles (MVs) and exosomes, which selectively transfer proteins, lipids, mRNA, and microRNA from one cell to another. While MVs are formed by extrusion from the plasma membrane, exosomes are a population of vesicles of endosomal origin that are stored within multivesicular bodies (MVBs) as intraluminal vesicles (ILVs) and are released when MVBs fuse with the plasma membrane (PM). In this sense, whether exosome biogenesis is entirely separate from ESCRT machinery or driven by it is still debated. In our laboratory, we found that the protozoan parasite *Giardia lambli*a, although lacking a classical endo-lysosomal pathway, can produce and release exosome-like vesicles (ElVs) in terms of size, shape, lipid, protein, and small RNAs composition. Our results indicated that ILV formation and ElV release are dependent on the ESCRT-associated AAA+-ATPase Vps4a and ceramide in this parasite. We also observed that the proteomic analysis of ElVs of genetic subtypes (assemblies) A and B, contain parasite-specific as well as EVs-conserved protein. Similar results were observed when we analyzed the RNA cargo. RNA sequencing analysis revealed that the ElVs of each assemblage contained distinct small RNA (sRNA) biotypes, suggesting a preference for specific packaging. These sRNAs were classified into three categories: ribosomal-small RNAs (rsRNAs), messenger-small RNAs (msRNAs), and transfer-small RNAs (tsRNAs), which may play a regulatory role in parasite communication and contribute to host-specificity and pathogenesis. The uptake experiments showed that ElVs were successfully internalized by the parasite trophozoites and that the RNAs were first located below the plasma membrane but then distributed along the cytoplasm. These investigations are part of the central objective of our project, which is to investigate whether there is information transmission between parasites that could define the role of EVs in drug resistance, pathogenicity, and cell survival of *G. lamblia*. **Supported by::** Agencia Nacional para la Promoción de la Ciencia y Tecnología, Argentina, grant number PICT2018-713 and PICT-2021-CAT-II-00073.
Keywords:: Extracellular vesicles ;Intercellular communication;Small RNAs (sRNAs).

CO-07 - **Characterizing the diversity of eukaryotes in the primate gut**

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Eukaryotes are a normal part of the mammalian gut ecosystem. They have historically been studied as parasites but high prevalence in healthy individuals suggest they may be indicators of a healthy gut ecosystem. Diversity in the gut is declining in response to modern lifestyles for both humans and non-human primates in captivity, but the consequences of these changes are not clear. I will present data from human populations and wild non-human primates that broadens our understanding of the diversity and variation of the eukaryome in health and disease. In a large cohort of children from Madagascar and Central African Republic (Afribiota), we find that the eukaryome is poorly correlated with clinical variables including chronic undernutrition, anemia, intestinal inflammation, and age. Interestingly, we find lower levels of the common gut protist Blastocystis in stunted children compared to non-stunted controls, mirroring findings that Blastocystis is less common in individuals with inflammatory or gastrointestinal disease compared to healthy individuals in other parts of the world. Across studies, we find high inter-subject variability, frequent co-occurrence of eukaryotes within an individual, and weak correlation with host factors. These patterns often contrast with those observed in the bacterial microbiome. Throughout, we use phylogenetic placement to refine taxonomic identification of gut eukaryotes and find only a handful eukaryotes that are likely residents of the gastrointestinal tract, while much of the signal from 18S rRNA gene metabarcoding data comes from transient organisms and from dietary and environmental sources. Our results highlight the importance of studying populations across the world to uncover common features of the eukaryome in health.

CO-08 - **RNA editing in non-model kinetoplastids**

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The kinetoplastids are unicellular flagellates that derive their name from the ‘kinetoplast’, a region within their single mitochondrion harboring its organellar genome of high DNA content. Some protein products of this mitochondrial genome are encoded as cryptogenes; their transcripts require editing to generate an open reading frame. This happens through RNA editing, whereby small regulatory guide (g)RNAs direct the proper insertion and deletion of one or more uridines at each editing site within specific transcript regions. An accurate perspective of the kDNA expansion and evolution of their editing across kinetoplastids has been difficult to achieve. Here, we resolved the kDNA structure and editing patterns in the early-branching kinetoplastid *Trypanoplasma borreli* and compare them with those of the well-studied trypanosomatids. We find that its kDNA consists of circular molecules of about 42 kb that harbor the rRNA and protein-coding genes, and 17 different (likely, linear) contigs of approximately 70 kb carrying an average of 23 putative gRNA loci per contig. Our analysis uncovered a putative gRNA population with unique length and sequence parameters that is massive relative to the editing needs of this parasite. In addition, we analyzed RNA editing in another non-model species, *Blastocrithidia nonstop,* a trypanosomatid species with all 3 stop codons reassigned to encode amino acids, and revealed that (with some background noise) it is limited to just 2 cryptogenes, *RPS12* and *COIII.*
We conclude that the organization of kDNA across known kinetoplastids represents variations on partitioned coding and repetitive regions of circular molecules encoding mRNAs and rRNAs, while gRNA loci are positioned on a highly unstable population of molecules that differ in relative abundance across strains. Likewise, while all kinetoplastids possess conserved machinery performing RNA editing of the uridine insertion/deletion type, its output parameters are species-specific.
Supported by:: GA CR 22-01026S **Keywords::** Trypanosomatidae;RNA editing;non-model species.

CO-9 - **Update of Malaria-associated Acute Respiratory Distress Syndrome**

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Malaria, still present in 84 malaria-endemic countries, caused 619,000 deaths in 2021. Different murine models are used to study severe malaria, such as cerebral malaria, hepatic and renal insufficiencies, placental malaria, severe anemia, and acute respiratory distress syndrome (ARDS), leading to the death of infected patients. This syndrome is characterized by acute inflammation, alveolar endothelium and pulmonary parenchyma injuries, dysfunction and increased permeability of the pulmonary alveolar-capillary barrier, and, consequently, edema formation. Thus, the lecture will address different aspects of the pathogenesis and the possibility of syndrome biomarkers. We observed that the integrity of the endothelial barrier is modified by the presence of erythrocytes parasitized with P. berghei ANKA, and there are changes in the actin, myosin, and microtubule cytoskeleton. In addition, results regarding the lungs and serum of mice with malaria-associated ARDS by quantitative proteomic analysis will address. In the lungs, 32 proteins were found upregulated only in mice that developed ARDS on days 7 and 9 dpi. Proteins involved in several processes, such as complement and coagulation cascade activation, neutrophil migration, extracellular matrix organization, and regulation of actin cytoskeleton, were found to be regulated, confirming preliminary results from our group. We characterize the expression of serum proteins in malaria-associated ARDS, especially acute phase proteins, to potentially identify early disease biomarkers. The integration of lung and serum proteomes data has been evaluating severe malaria with associated pulmonary complications that could spread a novel outlook on malaria-associated ARDS. **Supported by:**FAPESP 2020/03163-1 **Keywords:** malaria;acute respiratory distress syndrome;murine model.

CC-01 - **What we have learned about *Trypanosoma cruzi* endocytic pathway**

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Our group has been directly studying the endocytic pathway of *Trypanosoma cruzi* for 45 years. In this long way, we used the best tools we had to describe the macromolecule entry portals and the path they take to the compartment where they are stored, which we had called reservosomes. As the lysosomes are the last organelles of the endocytic pathway in all eukaryotic cells studied until now, we have also been looking for *T. cruzi* lysosomes, until we gave up and admitted that lysosomes are the reservosomes themselves. But not all of them... *T. cruzi* lysosome-like organelles are not homogenous, concerning their size, internal pH and enzymatic content, as mammal cell lysosomes are also not. In the last 10 years we have also contributed significantly to the structural knowledge of the cytostome-cytopharynx complex and its dynamics (and what dynamics!) in the epimastigote cell cycle and in the whole life cycle of *T. cruzi*. After many observations and quantifications, we can support that the cytostome is the main entry site for macromolecules in the proliferative forms, epimastigotes and amastigotes. But what about the flagellar pocket? Other trypanosomatids lacking the cytostome, such as *T. brucei* and *Leishmania sp* uptake molecules for nutrition and defense through the flagellar pocket, but in *T. cruzi*, records of a tracer at the flagellar pocket are extremely rare. On the other hand, we know very little about *T. cruzi* secretion pathways. Would the pocket membrane domain be a priority site of secretion? Or could macromolecules find their way out going upstream along the cytopharynx, as may be suggested by the acidic pH of its lumen and the proximity to the Golgi complex?
We urgently need secretory tracers and molecular markers to the *T. cruzi* endocytic compartments! Or another 45 years of observations... **Keywords**: Endocytosis;Cytostome;Flagellar pocket.

RT.01 – Immunity against protozoans

RT.01-01 - **Exhaustion on Leishmania amazonensis and *Leishmania braziliensis* infection**

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Chronic antigenic stimulation can lead to the induction of T cell exhaustion, which hampers the T cell response necessary for controlling the infectious process. In the case of infection with Leishmania amazonensis in mice, a suppressive capacity is observed, wherein the expression of PD-1 on lymphocytes and PD-L1 on macrophages, neutrophils, and dendritic cells contributes to the failure in generating an effective immune response. The infection by L. amazonensis triggers the expression of PD-L1 on these cells, indicating that the parasite exploits this pathway to evade the immune response. Treatment with anti-PD1 or anti-PD-L1 antibodies can control the parasite load by reinvigorating the CD4 + and CD8 + T cell responses and promoting increased production of interferon-gamma. On the other hand, infection with L. braziliensis exhibits the ability to induce an effective immune response capable of controlling the parasite load. However, when analyzing the infection by L. braziliensis, it is also observed that the parasite induces PD-L1 expression both in vitro and in vivo. Treatment with anti-PD1 and anti-PD-L1 antibodies in vivo enhances the control of the parasite load, indicating that the exhaustion process also occurs in Leishmania braziliensis infection. Furthermore, immunotherapy has the potential to improve the ability to control the parasite load by enhancing the interferon-gamma response. These findings support previous data obtained from patients with cutaneous leishmaniasis caused by Leishmania braziliensis and suggest the possibility of using immunotherapy in patients who do not respond well to conventional treatments.**Keywords**:Leishmania amazonensis;Leishmania braziliensis;exhaustion.

RT.01-02 - **Heme trafficking at the Leishmania-host interface**

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Parasites develop adaptations that allow them to acquire nutrients unidirectionally from their host to sustain their growth and reproduction. One such adaptation is the uptake of heme, an iron-containing organic ring that certain parasites cannot produce but is synthesized by all vertebrate hosts by a highly conserved multi-step pathway. This requirement for external heme is found in both parasitic nematodes and single-celled parasites, i.e, Trypanosoma and Leishmania. Thus drugs that target heme transport pathways unique to the parasite and not shared by its mammalian host have interesting therapeutic potential. We discovered HRG1, the first eukaryotic heme importer. HRG1 homologs exist across species but are genetically divergent, with only 13% identity between human HRG1 and LHR1, its homolog in Leishmania. We propose that Leishmania growth and virulence is inextricably intertwined with host heme and iron status as Leishmania is a heme auxotroph and resides within macrophages, the very cell that recycles body heme-iron. To study the relationship
between host heme status and parasitemia, we employ genetically-altered mouse infection models, ex vivo cell cultures, and functional assays in yeast. Results and implications from these studies will be discussed. Uncovering the role of essential nutrients on leishmaniasis could elucidate potential parasite-specific therapeutic targets that would limit host toxicity and improve quality of life.**Keywords:**Heme;Iron;Anemia.

RT.01-03 - **Regulation of Immunological Memory to *Toxoplasma gondii***

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Tallied up, parasites cause 96 million Disability Adjusted Life Years (DALYs) and 2 billion new infections every year. And yet, a fully protective vaccine to prevent any parasitic disease has eluded immunologists and parasitologists alike. Addressing why immunological memory fails and how vaccination can be used to prevent parasitic disease underpins the research in the Jensen lab. Specifically, we study requirements for heterologous immunity to highly virulent strains of the widespread intracellular parasite of animals, *Toxoplasma gondii*. We have identified parasite virulence factors that contribute to immune evasion and found that host genetics are major determinants of acquired immunity. In this seminar, I would like to discuss two genetic mapping experiments designed to understand mechanisms of acquired immunity to *T. gondii* that follow natural infection or vaccination. First, results from a large Collaborative Cross (CC) recombinant inbred mouse panel, which incorporates alleles from three sub-species of *Mus musculus*, revealed a significant contribution from a single locus centered on the Wnt-signaling pathway transcription factor, *Tcf7* or TCF-1. In resistant mice, enhanced central memory CD8 T cell responses and TCF-1 expression underlie the success of attenuated *T. gondii* vaccines against highly virulent South American strains. Second, results from a recombinant inbred (AXB;BXA) mouse panel revealed an unexpected role for B-1 cells and a regulator of NF-κB, *Nfkbid* or IκBNS, as paramount for humoral immunity to this parasite. Recently, we have found that antigens targeted by antibodies appear highly sensitive to the GPI-anchor, and when the GPI is modified in *T. gondii,* it significantly alters parasite virulence. In summary, we reason vaccination strategies aimed at *T. gondii* prevention, and likely other parasites, should target signaling pathways that activate the above transcriptional regulators to elicit appropriate immunological memory responses. Choice of immunogens will also be critical. Perhaps by including conserved biomolecules like the GPI, which is both an antigen and TLR agonist, heterologous immunity can be achieved against multiple parasite strains encountered in nature. **Supported by::** NIH **Keywords:**Forward Genetics;Vaccines;Toxoplasma gondii.

RT.01-04 - ***Trypanosoma cruzi* undermines the host immune response through purinergic signaling activation**

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Chagas disease, also known as American trypanosomiasis, is the primary chronic infectious heart disease worldwide and is caused by the intracellular parasite Trypanosoma cruzi. Following infection, both innate and adaptive responses control circulating parasite levels, but are insufficient to completely clear the infection. Therefore, one of the main challenges in understanding Chagas disease immunopathology is to find out why the parasite is not completely cleared, being able to sustain a pathological inflammatory environment that underlies the progression of cardiac lesions. After infection, the influx of immune cells consumes large amounts of oxygen, and ischemic cells rapidly respond to the hypoxic and inflammatory environment by releasing ATP. This extracellular ATP (eATP) triggers microbicidal immune responses but is quickly hydrolyzed to the potent immunosuppressive metabolite adenosine, mainly via the concerted activity of CD39 and CD73 ectoenzymes. We propose that CD73-derived adenosine has a central role in regulating immune response to T. cruzi infection. We have reported that pharmacological inhibition of CD73 activity early during the acute phase ameliorates the outcome of murine Chagas cardiomyopathy by preventing the shift of cardiac type-M1 to anti-inflammatory M2 macrophage that occurs at 7 days postinfection (dpi), enhancing nitric oxide (NO) production, and diminishing the frequency of IL-4- and

IL-10- producing CD4+ T-cells. In concert, CD73 abrogation decreased the local parasite burden and improved cardiac functionality. In accord, predominant presence of T-cells in Chagas patient myocardium that correlated with the number of CD73-expressing cells and the presence of HIF-1α+ cells were also determined. In contrast, circulating T-lymphocytes display reduced expression of CD39 and CD73 ectoenzymes in chronically infected individuals, associated with increased plasmatic ATP levels compared with non-infected donors. Thus, it is plausible that in the periphery, T-cells fail to promote eATP degradation and an inflammatory microenvironment, but in the myocardium, T-lymphocytes upregulate the enzymatic machinery to enhance ATP metabolization fostering a regulatory milieu. The results evidence that T. cruzi subverts the immune response to infection through purinergic signaling activation in experimental and human Chagas disease. **Supported by::** R01 (RAI176457A); SECyT-UNC; Agencia I+D+i-FONCyT; CONICET
Keywords: Chronic Chagas cardiomyopathy;lymphocytes;CD73; adenosine.

RT.02 – Protozoan Vectors and Viruses

RT.02-01 - **Identification and characterization of new viruses in *Rhodnius prolixus***

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Hemimetabolous insects of the subfamily Triatominae account for more than 150 species, including *Rhodnius prolixus* and *Triatoma infestans*, two hematophagous species firmly associated with the transmission of Chagas disease. The etiologic agent of the parasitosis is the protozoan *Trypanosoma cruzi*, which is transmitted to the human host during the blood meal. Despite the medical relevance, the range of viral species infecting Triatomine insects remains largely unexplored. In stark contrast with mosquitos and other insects,where the host/virus interaction has been investigated in much detail, our knowledge of triatomine viruses mostly relies on the characterization of Triatoma virus (TrV). TrV proved to be a triatomine-specific entomopathogenic virus causing leg paralysis and death in triatomine colonies. Very recently, we identified and partially characterized seven new viruses in Rhodnius prolixus using metatranscriptomic approaches. *Rhodnius prolixus* viruses 1-7 (RpV1-7) can be classified in three different families: Iflaviridae (RpV1 and 2), Permutotetraviridae (RpV3, 4 and 7) and Solemoviridae (RpV5 and 6). Interestingly, the putative RpV2 polyprotein shares a high degree of aminoacid sequence identity with that of Slow Bee Paralysis Virus, which is responsible for the collapse of bee hives and huge economic losses. All the viruses display single-stranded positive sense RNA genomes and are maintained in the insect population via transovarial vertical transmission and coprophagy. Different from TrV however, the RpVs do not show apparent effects neither on the viability nor on the fertility of the insects. We found that an antiviral system centered on viral small interfering RNAs is active in Rhodnius and might explain at least in part these findings. Finally, we asked whether the RpVs can infect *Trypanosoma cruzi*. In a recent study, it was shown that enveloped and non-enveloped viral-like particles can be observed in the cytoplasm of *T. cruzi* by electron microscopy. In agreement with these data, we found that the RpVs can infect T. cruzi and, at least RpV1, is detectable in the cell cytoplasm. Furthermore, our electron microscopy assays reveal viral-like particles in Rhodnius'; gut, thus suggesting that *T. cruzi* can be infected in nature while passing through the host intestine. Our results start to shed light on the complexity of the triatomine virome and its ternary interaction with the insect and the protozoan *T. cruzi*.
Keywords: Rhodnius;virus;Trypanosoma.

RT.02-02 - **Identification of endogenous Viral elements (EVEs) in embryonic cell lines and insects of *L. longipalpis* and others phlebotomine sandflies from Brazil**

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The phlebotomines of the genera *Phlebotomus* and *Lutzomyia* are the main vectors of leishmaniasis in the Old and New World, respectively. In addition to vectors of leishmaniasis, the phlebotomines of the Old World are known to be vectors of several arboviruses. Little is known about the vectorial capacity of New World sandflies in the transmission of viruses to humans. Our group has conducted various studies with Lutzomyia longipalpis, Brazil's main vector of visceral leishmaniasis. One of the main models used for *L. longipalpis in vitro* studies is the LL-5 embryonic cell line. These cells, when transfected with double-stranded RNAs, show non-specific antiviral activity and the conditioned medium of these cells induces this antiviral phenotype in non-transfected cells. Transcriptomic analysis of this conditioned medium revealed the presence of RNAs that encode for different Rhabdovirus proteins. Rhabdoviruses are RNA viruses, so it these sequences were expected to be identified in cDNA samples from LL-5 cells and, potentially, in samples of adult insects. DNA samples were also used in PCRs as a negative control since these viruses never appear in the form of DNA during their replicative cycle. Surprisingly, these elements were also identified in these DNA samples, indicating insertion into the genome. The presence of these endogenous viral elements (EVEs) was confirmed by *in silico* and *in vitro* studies.
The presence of EVEs sequences integrated into the genome of vertebrates, invertebrates, and plants is described in various works. Depending on the role they play in the host along time EVEs, can be eliminated or persist during the evolutionary process. Some EVEs may exert beneficial functions for their hosts in a process known as exaptation. A classic example the transcription and/or translation of these EVEs by the host itself leading to resistance to viral infections.
In this project, we also investigated the presence of these inserts in different sand flies populations in Brazil. **Supported by::** CNPq, Faperj **Keywords:**Endogenous Viral Element;Lutzomyia longipalpis ;RNA.

RT.02-03 - **Insights into the Interaction between protozoan parasite vectors and Viruses**

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Studying viral agents within vectors such as Rhodnius and Lutzomyia holds paramount significance due to their profound implications for public health and the transmission dynamics of diseases. These arthropod vectors assume pivotal roles in disseminating parasitic infections, such as Chagas disease and leishmaniasis, which exert a substantial global burden. Elucidating the virological infections within these vectors offers insights into potential co-infection scenarios and their synergistic ramifications on disease manifestations. Furthermore, delving into viral interactions provides opportunities to advance targeted control methodologies that can curtail vector populations and disrupt disease transmission cycles. Thus, we employed advanced small RNA deep sequencing techniques on specimens derived from Lutzomyia longipalpis and Rhodnius prolixus. Our primary objective was to discern the presence of viral entities and their plausible interactions with the insect immune systems. In the context of Lutzomyia longipalpis, our investigations led to the identification of six novel viruses. These viruses exhibited diverse profiles of virus-derived small RNA, with some indicative of RNA interference (RNAi) pathways targeting, while others suggested potential RNA degradation patterns possibly resulting from accumulation, implying inhibition of the siRNA pathway. Notably, we examined the non-coding small RNA response of L. longipalpis following artificial infections with Vesicular stomatitis virus (VSV), a virus naturally

propagated by this phlebotomine in the wild. This analysis unveiled a heightened production of VSV-derived siRNAs, accompanied by the modulation of host-derived miRNAs. Additionally, virus-derived small RNAs exhibited different profiles for specific species. Moreover, leveraging small RNAs aligned to putative viral sequences enabled successful discrimination between endogenous and exogenous elements. For Rhodnius, a similar approach involving deep small RNA sequencing disclosed the presence of new viruses spanning at least five families: Permutotetraviridae, Iflaviridae, Sobemoviridae, Narnaviridae, and Partitiviridae. Through an oxidation-based strategy, we investigated the association of virus-derived small RNAs with Argonaute proteins. In both Rhodnius and Lutzomyia cases, a robust small RNA-mediated response against viral agents was evident, implying that the RNAi pathway is involved in the immune response of these protozoan parasite vectors against viral infections.

In conclusion, the comprehensive exploration of viral agents and their interplay with antiviral pathways within these vectors stands as a pivotal stride towards enhancing our capacity to mitigate the repercussions of vector-borne illnesses. This endeavor safeguards human and animal health while fostering the development of more efficacious and sustainable interventions within the realm of public health.

Keywords: Virus infection, protozoan parasite vectors;Lutzomyia;Rhodnius; RNAi pathway.

RT.03 – Fascinating peculiarities regarding protozoan parasites

RT.03-01 - **Uncovering the Mechanisms of VSG mRNA Regulation through Multi-Omics Analysis**

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Trypanosoma brucei causes sleeping sickness in humans and nagana in cattle. The unicellular parasite is transmitted by the bloodsucking tsetse fly. In the mammalian host's circulation, the parasite avoids the host immune response by periodically replacing a monolayer of variant surface glycoproteins (VSG) that covers its cell surface. Such antigenic variation is a key pathogenesis mechanism that enables *T. brucei* to establish long-term infections. VSG is expressed exclusively from subtelomere loci in a strictly monoallelic manner, and DNA recombination is an important VSG switching pathway. VSG mRNA accounts for nearly 10% of total mRNA, and its high stability is essential for trypanosome survival. To determine the mechanism by which VSG mRNA stability is maintained, we used mRNA affinity purification to identify the proteins specifically associated with the VSG mRNP. By purifying the VSG mRNA, we have recently identified Cyclin F-Box 2 (CFB2) as the protein responsible for VSG mRNA stability in bloodstream forms. As occurs in the active VSG KD, we observed that depletion of CFB2 leads to decreased levels of active VSG transcripts and a cytokinesis arrest. CFB2 recognizes a conserved 16mer element that is found in the 3'-UTRs of all VSG mRNAs. Moreover, we could demonstrate the mechanism by which CFB2 acts: recruitment of a stabilizing complex that includes MKT1, PBP1, PABP2, and the cap-binding translation initiation complex EIF4E6/G5. We have now validated additional candidates from this VSG mRNA-bound proteome and identified a novel protein as a regulator of the VSG switching pathway. RNA-seq analysis indicated that this novel protein is important in maintaining the level of active VSG mRNA, similarly to the CFB2 depletion. But unlike the CFB2 KD, its depletion did not induce a cytokinesis arrest. Surprisingly, its depletion caused a rapid increase in the levels of phosphorylated H2A and replication protein A1 (RPA1), proteins implicated in DNA double-strand break (DSB) repair. More notably, this led to a dramatic increment in the VSG switching rate (~150-fold up) with switchers arising predominantly by recombination processes. Reflecting this phenotype, we named this gene 'Suppressor of VSG Switching 1 (SVS1)'. Our findings suggest the existence of a potential interaction

between VSG mRNA surveillance and VSG switching in this parasite. We are currently investigating the mechanisms underlying the role of SVS1 to gain a deeper understanding of how VSG switching and the regulation of monoallelic VSG expression are controlled. Beyond *T. brucei*, the mRNP purification approach has the potential to supply detailed biological insight into metabolism of relatively abundant mRNAs in any eukaryote organism. **Supported by**:: NIH, AGENCIA I+D+i
Keywords:VSG;mRNA stability;antigenic variation.

RT.03-02 - **The chromatin and protein acetylation in *Toxoplasma gondii***

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Toxoplasma gondii is an obligate intracellular parasite and the causative agent of toxoplasmosis. In Brazil, this disease can reach 60% of incidence, depending on the region, culture, and socioeconomic conditions. Although usually asymptomatic, toxoplasmosis is extremely dangerous when acquired during pregnancy or in immunocompromised patients and can result in severe damage. In Brazil, it is one of the main causes of severe eye damage, including blindness. In Brazil, the disease shows more severe symptoms than those observed in the northern hemisphere, which indicates that the strains found in our territory are more virulent when compared to those found in the USA and Europe. The parasite's life cycle passes through sexual stages in felines and asexual stages in intermediate hosts, including humans. These transitions require fine control of gene expression. At the center of this regulation are the histones. Toxoplasma has four core histones, with high similarity with human histones. However, the presence of a histone linker H1 was still a question. We identified a small and basic protein (TgH1-like) that forms a complex with most histones. The knockout of tgh1-like resulted in fewer peripheral heterochromatin and unexpected asynchronous division. Histones are subject to posttranslational modifications, and according to ToxoDB, TgH1-like has phosphorylation and ubiquitylation sites, which were mutated and confirmed the problem during division, showing those modifications are essential for the correct cell cycle. As histone, H1-like, all histones, and other proteins are targets for several post-translation modifications. One of the most abundant is acetylation which can affect chromatin compaction and other functions. Toxoplasma has five classic HDACs, and we started characterizing those with unique characteristics: TgHDAC2 and TgHADC4. TgHDAC2 has around 200 amino acids inside the HDAC domain, specific to some members of the *Apicomplexa phylum*. The knockout of tghdac2 showed defects in virulence and replication. Structure analyses suggested that these unique sequences are more related to function than stability. The location of TgHDAC2 surrounds the daughter cells. TgHDAC4, a class IV histone deacetylase, is the most understandable class of deacetylases. By phylogenetic analysis, we observed that TgHDAC4 group with prokaryotic HDACs. TgHDAC4 is located in the apicoplast, a plastid-like structure with secondary endosymbiotic origin. TgHDAC4 is immunoprecipitated with proteins in the apicoplast and apparently is unrelated to the apicoplast's DNA. Several attempts to delete the protein suggest it is essential. We currently use the TeT system to analyze the TgHDAC4 function by condition KO. HDACs are known and proven inhibitor targets for several diseases. Understanding how Toxoplasma's HDACs work could result in a potential target for better treatments for toxoplasmosis. **Keywords:**Toxoplasma;histone;histone deacetylases.

RT.03-03 - **Synthesis and translation of selenocysteine in Kinetoplastida and Bacteria**

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The genetic code, composed of 64 different codons and called “Universal”, codes for 20 amino acids. This number has already been modified to include two new amino acids, selenocysteine and pyrrolysine, using the same repertoire of 64 codons. Selenium, in particular, exists naturally in organic forms such as selenomethionine and selenocysteine and in various inorganic forms such as selenites, selenates and others. Selenium, instead of sulfur, in the form of the amino acid selenocysteine (Sec-U) results in increased catalytic activity of selenoenzymes as a result of Se being more nucleophilic and being ionized at physiological pH. The selenocysteine synthesis pathway and the respective genes for SELB, SELD, PSTK, SecSepS and tRNASerSec (SelC) have been identified in protozoa. The functional and structural characterization of the enzymes involved in the synthesis of selenocysteine, as well as the investigation of the physiological function of this pathway in protozoa, parasites or not, are the objectives that may contribute to the identification of new molecular targets and in the elucidation of the way in which the organisms evolve. recognition mechanisms between tRNA and proteins. **Keywords**: Selenocysteine;Translation;Kinetoplastida.

RT.03-04 - **Total parasite biomass but not peripheral parasitaemia is associated with endothelial and haematological perturbations in Plasmodium vivax patients**

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Plasmodium vivax is the major cause of human malaria in the Americas. How *P. vivax* infection can lead to poor clinical outcomes, despite low peripheral parasitaemia remains a matter of intense debate. Estimation of total *P. vivax* biomass based on circulating markers indicates existence of a predominant parasite population outside of circulation. In this study we investigate associations between both peripheral and total parasite biomass and host response in vivax malaria. We analysed parasite and host signatures in a cohort of uncomplicated vivax malaria patients from Manaus, Brazil, combining clinical and parasite parameters, multiplexed analysis of host responses and *ex vivo* assays. Patterns of clinical features, parasite burden and host signatures measured in plasma across the patient cohort were highly heterogeneous. Further data deconvolution revealed two patient clusters, here termed Vivax^{low} and Vivax^{high}. These patient subgroups were defined based on differences in total parasite biomass but not peripheral parasitaemia. Overall Vivax^{low} patients clustered with healthy donors and Vivax^{high} patients showed more profound alterations in haematological parameters, endothelial cell (EC) activation and glycocalyx breakdown and levels of cytokines regulating different haematopoiesis pathways compared to Vivax^{low}. Vivax^{high} patients presented more severe thrombocytopenia and lymphopenia, along with enrichment of neutrophils in the peripheral blood and increased neutrophil-to-lymphocyte ratio (NLCR). When patients’ signatures were combined, high association of total parasite biomass with a subset of markers of EC activation, thrombocytopenia and lymphopenia severity was observed. Finally, machine learning models defined a combination of host parameters measured in the circulation that could predict the extent of parasite infection outside of circulation**.** Altogether, our data show that total parasite biomass is a better predictor of perturbations in host homeostasis in *P. vivax* patients than peripheral parasitaemia.

This supports the emerging paradigm of a *P. vivax* tissue reservoir, in particular in the hematopoietic niche of bone marrow and spleen.**Keywords**:Plasmodium vivax, malaria parasite;total biomass, tissue infection;endothelial activation, haematopoiesis.

RT.04 – Current Topics in Protozoology
(organized by the Early Career Research (ECR) committee)

RT.04-01 - **Modeling of epidemiological parameters in vector-borne diseases**

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Introduction. Vectorial transmission of *Trypanosoma cruzi* by *Triatoma infestans* was eliminated from Brazil. However, the vertical mode of transmission, congenitally through a carrier mother to her baby, is the disease's main mechanism of persistence. The absence of a specific surveillance data system determines that prevalence and congenital transmission rates remain unknown. This makes harder to establish public policies and actions for the control and elimination. This study aimed to estimate the epidemiological parameters of Chagas disease in municipalities in Brazil. **Methods**. A meta-analysis was carried out to extract data on prevalence in specific population groups, in municipalities in Brazil, 2010–2022. Indicators available in the information systems were selected at municipal scale. Statistical modeling of extracted data from meta-analysis as a function of those obtained from information systems was applied using maximum likelihood and the principle of parsimony. **Results**. The 5 best models were selected, from a total of 549 tested models, to obtain a consensus model (adjusted R^2=54%). The most important predictor was from the primary care information system. The mean prevalence of the disease was estimated at 3.4% (±2.9%) in 3,662 municipalities in Brazil. Number of Chagas disease carriers was estimated in the general population (~3.9 million), women (~2.2 million) and women of childbearing age (~630 thousand). The calculated disease reproduction rate was 1.035. All estimates refer to 2015–2016. **Conclusions**. Estimated prevalence of Chagas disease in women of childbearing age is presented as an indirect estimate of the risk of vertical transmission of Chagas disease by municipality in Brazil. The estimated values are in line with those predicted by PAHO/WHO and mathematical projections. It is proposed to use this estimate in reference municipalities for a pilot project of the national pact for the elimination of congenital Chagas disease. **Supported by**:: São Paulo Research Foundation (FAPESP) grant number 21/06669-6 **Keywords:**Pregnant Women;Vertical Transmission of Infectious Diseases;Trypanosoma cruzi.

RT.04-02 - **Integrative systems biology of host-Plasmodium interactions**

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Although the burden of malaria has decreased in the last decades, the disease still affects millions of individuals and causes thousands of deaths worldwide. The factors that drive protection or pathology after infection with *Plasmodium* are still not completely solved. Systems biology emerged from changes in scientific philosophy moving from a reductionist point of view towards a holistic understanding of the whole biological systems under assessment. High-throughput technologies coupled to rapid development of computational biology spurred studies about molecular profiles of patients, controlled human malaria infection, and non-human primate models. The first

studies using these technologies have been reported almost over two decades, but limited sample sizes, lack of generalizability and requirement for robust and independent validation prevent the translational potential of findings from individual studies. We sought to use an integrative systems biology approach to analyze data from diverse and distinct cohorts and experimental models to gain novel insights into the molecular mechanisms of host-*Plasmodium* interactions. We used these data in three different studies which will be summarized here. The first study aimed to identify conserved transcriptional signatures of individuals with malaria. For that, we integrated gene expression data obtained through microarray of RNA sequencing technologies from over 800 samples. Samples were split into discovery and validation cohorts. We found a 16-gene signature composed of molecules involved in host defense (*C1QA*, *GBP1*) and metabolism (*SMPDL3A*), denominated the Malaria Meta-Signature (MMS). ROC analysis revealed that the MMS discriminates individuals with malaria from healthy controls with high-performance in discovery (AUC = 0.98) and validation (AUC = 0.97) cohorts. The MMS also distinguishes from asymptomatic *Plasmodium* infection and other febrile and inflammatory diseases. The MMS correlates with parasitemia, RBC counts and IL-1RA, IL-6, IL-10, CXC10, CCL4 in the plasma. We validated the functional of *SMPDL3A* by integration with metabolomics data, which revealed a positive correlation between *SMPDL3A* expression and levels of adenosine monophosphate (AMP) in the plasma. These findings demonstrate a robust transcriptional biomarker signature of malaria and suggest the activity of unknown metabolic regulators of the inflammatory response. In a following study, we compiled 9 datasets of dual RNA sequencing data from both malaria patients and *P. falciparum* (Pf) to identify robust associations between the transcriptional response of hosts and parasites. We reduced the dimension of data from human gene expression to 346 blood transcription modules (BTM) that reflect immune and metabolic processes in the blood. We evaluated the correlations between BTMs and Pf genes with Spearman correlation and retained associations with $p < 0.001$. Associations found in approximately 70% of the datasets (6 datasets) formed a network with 60 nodes (BTMs and Pf genes) linked by 30 edges. For example, we found interactions between RIG-I-like receptor signaling and Pf genes such as *RESA*, *KIC6,* and *HMGB3*. BTMs reflecting TLR and inflammatory signaling as well as CCR1, 7 and cell signaling we also associated with Pf *HMGB3* expression. These findings demonstrate unknown concerted activity between host and Pf gene expression and suggest novel molecules involved in innate immune activation. In a third study, we collected multiple datasets of plasma metabolomics from individuals with malaria and healthy controls. We performed an integrated analysis that revealed metabolic pathways that are commonly disrupted with malaria for independent and diverse cohorts. Taken together these studies demonstrate that multi-omics data repurposing offers effective opportunity to discover molecular features of host-*Plasmodium* interactions**.** **Supported by**:: Instituto Serrapilheira, FUNAPE, CNPq **Keywords**:Malaria;Systems Biology;Multi-omics technologies.

RT.04-03 - **Unraveling the mechanisms of endocytosis by intracellular amastigotes of *Trypanosoma cruzi***

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Endocytosis is a vital cellular process that involves the uptake of molecules and particles from the extracellular environment. It also plays a significant role in removing plasma membrane components and modulating cell signaling. In *Trypanosoma cruzi*, the protozoan parasite responsible for Chagas disease, endocytosis is crucial for various aspects of its lifecycle. During the epimastigote stage, present in the midgut insect vector, the parasite can endocytose a wide range of macromolecules through the cytostome-cytopharynx complex. These macromolecules are then delivered to endosomal compartments and reservosomes, which are lysosome-like organelles. The endocytosis of macromolecules supplies the cell with nutrients for replication, and starvation act as a trigger for differentiation into infective metacyclic trypomastigotes. The clinically relevant amastigote stage, which develops intracellularly in the vertebrate host, reside in the host cell cytosol. They have access

to the host's cytosolic macromolecules and organelles. Although amastigotes possess a functional endocytic pathway, limited information is available regarding the macromolecules they can endocytose and their interactions with host organelles, from where they could scavenge molecules. Cholesterol performs most of the sterol found in intracellular amastigotes suggesting that they can scavenge cholesterol from the host cell. We investigate cholesterol traffic in infected host cells by using fluorescent cholesterol tracer, confocal and super-resolution microscopic analysis, and high-resolution electron microscopy. Our data suggests a participation of ER contact sites as platforms for cholesterol transfer to the parasite. Understanding the mechanisms of endocytosis in amastigotes is crucial for unraveling the intricate interplay between the parasite and the host cell, providing insights into the parasite's survival strategies and nutrient acquisition. The deciphering these mechanisms will lead to a valuable knowledge to develop targeted therapeutic interventions and enhance our understanding of Chagas disease. **Supported by::** Faperj; CNPq **Keywords:**amastigotes;endocytosis ;contact-sites.

RT.04-04 - **What are trypanosomatids presumably exclusive to insects doing in human hosts? A closer look at isolation of *Crithidia* parasites from visceral leishmaniasis patients in Sergipe, Brazil.**

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Reports about occurrence of monoxenous trypanosomatid infections in humans have been gradually increasing. By analyzing clinical isolates (CIs) from visceral leishmaniasis (VL) patients in Sergipe, Brazil, we have found that 51 out of 62 CIs are phylogenetically related to *Crithidia fasciculata*. In view of these surprising findings, we have endeavored to study these new parasite strains, integrating genomic and phenotypic approaches to characterize them. For this, we performed whole-genome sequencing (WGS) analysis of *Crithidia sp* LVH60A strain and assembled the genome in 38 chromosomes. Genome alignment between LVH60A and *C. fasciculata* revealed an average nucleotide identity (ANI) of 93%, which is low to be considered within of a same species. Also, we performed WGS analysis of another 47 *Crithidia* CIs from VL. Read mapping rate of the 47 CIs to *C. fasciculata* was ~72%, whereas to LVH60A genome was ~ 99%. Cell growth at 25°C of LVH60A was similar to *C. fasciculata* (doubling-time of 9.6 and 7.81 h, respectively), whereas *L. infantum* doubling time was 15.2 h. Cell growth at 35°C of LVH60A was similar to *L. infantum*, corroborating its thermotolerance feature. Morphological analyses of cultured parasites were performed using scanning and transmission electron microscopy. *In vitro* infections were performed using murine and human cell lines and parasite load analyzed at post infection periods. The average rate of infection for *Crithidia sp* was 27%, whereas for *L. infantum* and *C. fasciculata* were 40% and 5%, respectively. In addition, dual RNA-seq analyses of human macrophages infected with different *Leishmania* and *Crithidia* strains during time-course infection will help to identify transcriptomic changes in the host and the parasite, assessing the differences between each type of parasite infection. We hope that the study of this new parasite will provide insights in the evolution of parasitism in Trypanosomatidae, besides understanding this new infection in humans. **Supported by**:: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP): 2016/20258-0 **Keywords**:Human Visceral Leishmaniasis;Crithidia sp LVH60A;whole-genome sequencing.

RT.05 – Progress in Vaccine Development and Diagnostic tests for parasitic diseases

RT.05-01 - *Toxoplasma gondii* vaccines: The past, present, and future

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Toxoplasma gondii is a protozoan parasite with worldwide distribution, which may be found in animals from the arctic, rain forest, arid zones, and even in marine mammals. Toxoplasma gondii normally causes a subclinical infection in most animal species, however, a primary infection during pregnancy can cause fetal pathologies, as well as abortions in humans and some animal species. . Additionally, in spite of the highly effective antiretroviral therapy (HAART) there is an estimated incidence of toxoplasmic encephalitis (TE) occurring in 16% of human immunodeficiency virus infected patients. Ocular toxoplasmosis (OT) is another concern with *T. gondii* infection and this may occur following congenital and acquired transmission, and the risk of OT is highly variable depending on geographic region, ranging from 2% (Europe and North America) to 18% (southern Brazil). Moreover, there are reports that *T. gondii* may be associated with psychiatric disorders, and may affect human behavior, personality and other phenotypic traits.
The main infection sources for human beings are through consumption of either vegetables or water contaminated with sporulated T. gondii oocysts, and undercooked meat infected with tissue cysts. Therefore, based on the longevity of tissue cyst in pork, which can remain more than two years, and that pork is one of the most important sources of T. gondii infections for humans, developing a vaccine against *T. gondii i*n pigs would be very desirable, and focused on tissue cyst reducing. Another important factor in the epidemiology of toxoplasmosis is the definitive host. Cats assume an important role due to the close interaction with human beings; infected cats shed millions of oocysts in the feces that contaminate the environment. The risk of infection via sporulated oocysts in human populations has been well documented. These data demonstrate the need to control oocyst shedding by cats, however, few studies have been conducted with this aim. Treatments are available to reduce clinical signs but there are no drugs available that kill the parasite or cure the host from infection. There is just one commercial vaccine available (Toxovax®, MSD Animal Health) that is used in United Kingdom, New Zealand, France, and Ireland. This vaccine comprises live tachyzoites of the incomplete S48 strain that has lost the ability to differentiate into tissue cysts in animals. The vaccine is licensed only for use in sheep and goats to be administered prior to mating, however, there are some concerns about its safety as the vaccine can infect humans and as it is a live vaccine it does have a short shelf life. Others live vaccines (RH, and T263) have shown protection against toxoplasmosis, but these carry the risk of reverting to virulence. Data obtained from Medline databases (PubMed, NCBI) using the key words "*Toxoplasma gondii*"; and "vaccine";, focusing on the years 2019, 2020, 2021, 2022, and 2023 (until July 2023) revealed that approximately 366 articles addressing vaccines against *T. gondii* were published, 80 in 2019 , 83 in 2020 , 86 in 2021 , 74 in 2022, and 43 in 2023. Most of these studies used mice (50 %, 184/366) as an experimental model, two used sheep, two in pigs, and three in cats. An important factor in *T. gondii* is that the main route of infection of the host is oral, and oocysts are the main form of infection for herbivores, and tissue cysts are a route of infection for pigs and people, so the local immunity in the gut via lymphocytes (mainly intraepithelial lymphocytes and IgA) are of fundamental importance in host resistance to the parasite. The focus of this presentation is to present the past, present, and future of the epidemiology, biology, parasite diversity and host-parasite interactions of *T. gondii* and to review current vaccination strategies further to the recent papers and the prospects for future vaccines against this parasite. **Keywords:**Toxoplasmosis;livestock, cats, pigs;animals, humans.

RT.05-02 - **Non-clinical toxicity and immunogenicity evaluation of repeated administration of a *Plasmodium vivax* malaria vaccine in mice and rabbits**

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Malaria caused by Plasmodium vivax is a pressing public health problem in tropical and subtropical areas. However, little progress has been made toward developing a *P. vivax* vaccine, with only three candidates being tested in clinical studies. We previously reported that one chimeric recombinant protein (PvCSP-All epitopes) containing the conserved C-terminus of the *P. vivax* Circumsporozoite Protein (PvCSP), the three variant repeat domains, and a Toll-like receptor-3 agonist, Poly(I:C), as an adjuvant (polyinosinic-polycytidylic acid, a dsRNA analog mimicking viral RNA), elicits strong antibody-mediated immune responses in mice to each of the three allelic forms of PvCSP. In the present study, a non-clinical safety evaluation was performed to identify potential local and systemic toxic effects of the PvCSP-All epitopes combined with the Poly-ICLC (Poly I:C plus poly-L-lysine, Hiltonol®) or Poly-ICLC alone subcutaneously injected into C57BL/6 mice and New Zealand White Rabbits after a 21-day recovery period. Overall, all observations were considered non-adverse and were consistent with the expected inflammatory response and immune stimulation following vaccine administration. Vaccine-induced antibody responses and vaccine-specific antibodies were detected in developmental toxicity studies in mice and rabbits. Mice that received the vaccine formulation were protected after the challenge with *Plasmodium berghei* sporozoites expressing CSP repeats from *P. vivax* sporozoites (Pb/Pv-VK210). In conclusion, in these non-clinical models, repeated dose administrations of the PvCSP vaccine with a Poly-ICLC adjuvant were immunogenic, safe, and well tolerated. **Supported by::** FAPESP (2012/13032-5) and INCTV

RT.05-03 - **Exploring miRNAs as potential biomarkers using an experimental model of chronic Chagas disease cardiomyopathy**

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MicroRNAs are small non-coding RNA molecules that play a crucial role in post-transcriptional gene regulation. They have been implicated in various biological processes, including development, differentiation, and disease pathogenesis. Emerging evidence suggests that miRNAs are involved in the pathogenesis of Chagas disease (CD), being associated with key processes, such as immune response modulation, cardiac remodeling, and parasite persistence. During the chronic phase of CD, miRNAs have been implicated in the regulation of cardiac remodeling processes, including fibrosis, hypertrophy, and apoptosis. Altered miRNA expression profiles have been observed in the hearts of Chagas disease patients, suggesting their involvement in the progression of cardiac pathology.
We investigated the miRNA transcriptome profiling in the cardiac tissue of chronically *T. cruzi*-infected mice treated with a suboptimal dose of benznidazole (Bz), the immunomodulator pentoxifylline (PTX), or the combination of both (Bz+PTX), following the CCC onset. At 150 days post-infection, Bz, PTX, and Bz+PTX treatment regimens improved electrocardiographic alterations when compared with the vehicle-treated animals. miRNA Transcriptome profiling revealed considerable changes in the differential expression of miRNAs in the Bz and Bz+PTX treatment groups compared with the control group. We observed that *T. cruzi* infection induced the upregulation of miR-146b-5p expression in the heart tissue of chronically infected mice and in *in vitro* cultivation of rat cardiomyoblasts (H9C2 cells), which was reversed upon Bz and Bz+PTX treatment regimens. Understanding the role of miRNAs in Chagas disease could have important implications for the development of novel diagnostic and therapeutic strategies. By targeting specific miRNAs, it may be possible to modulate immune responses, prevent cardiac damage, and interfere with parasite persistence.
Supported by:: CNPq, FAPERJ, Fiocruz (INOVA**)** **Keywords**:Chagas disease cardiomyopathy;Micro RNAs;Biomarker.

RT.05-04 - **LeishID: LAMP-based molecular diagnostic tool for species-specific *Leishmania* detection**

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Point-of-care (PoC) molecular differential diagnostic of leishmaniasis is urgently need since it can be misdiagnosed for example as sporotrichosis. Additionally, recent reports reveal the presence of dermotropic-associated species causing visceral leishmaniasis (VL) and vice-versa. Thus, a test able to concomitantly identify the *Leishmania* species is important to better understand epidemiology and physiopathology. In this regard, we developed the LeishID, a LAMP-based molecular tool able to detect small quantities of parasite DNA and differentiate among *L. amazonensis*, *L. infantum* and *L. braziliensis*. The probes were designed based on a *Leishmania* pangenome approach, where we selected species-specific DNA sequences filtered on the accessory genome. The LAMP reaction can be detected by naked eye, since the colorimetric output is based on the buffer acidification, leading to a yellow color, during DNA amplification in the positive test. Depending on the target, positive reactions can be detected as soon as 15 min at 65 ºC. However, 40 min incubation, increases test sensitivity. The LAMP test was able to detect as low as 1 pg of extracted *Leishmania* DNA for all tested species. Species-specific sets of primers were able to detect the species they were designed for without cross-reactivity among them neither on mammalian DNA. Clinical validation using spleen biopsies of dogs with VL, samples derived from skin lesions of cutaneous leishmaniasis from human patients and Phlebotominae sandflies, revealed sensitivity varying from 93-97% and specificity of 97-10%. Thus, LeishID is a PoC compatible solution, rapid, specific, and sensitive to differentiate *Leishmania* species in clinically relevant concentrations.
Supported by:: Fundação de Amparo à Pesquisa do Estado de Minas Gerais - Fapemig (RED-00032- 22) e Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq - (312965/2020-6) **Keywords:**Leishmania;LAMP;pangenome.

RT.06 – Genomics

RT.06-01 - **Genomes of *Endotrypanum schaudinni* and *Zelonia costaricensis*: Expansion of multigene families in Leishmaniinae parasites that are close relatives of *Leishmania* spp.**

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The Trypanosomatidae family consists of obligate parasites of a range of organisms, including vertebrates, invertebrates, and plants. As such, genomic plasticity is an essential capability in order to adapt to new hosts and conditions. The Leishmaniinae subfamily is especially interesting in that it presents lineages that are either monoxenous or dixenous parasites, enabling comparative studies aiming towards a better understanding of the evolution of parasitism and lifestyle. Here, we focus on *Zelonia costaricensis* and *Endotrypanum schaudinni*, considered some of the nearest relatives of the genus *Leishmania*, an important parasite to humans. Parasite genomes evolve by employing gene repertories that can expand (gain genes) or contract (lose genes) depending on environmental pressures, which, in the case of the trypanosomatids, is their host (or hosts). We have used comparative genomics methods to identify ortholog groups (OGs) shared among 27 different trypanosomatid genome, then selecting OGs that were specific of our taxa of interest. The *E.*

schaudinni and *Z. costaricensis* genomes were assembled and display sizes of 29.9 Mb and 38.0 Mb, with 9,711 and 12,201 protein-coding genes predicted, respectively. Furthermore, *E. schaudinni*, a dixenous parasite, displayed a high number of multicopy genes, which include for example gp63 and gp46. Conversely, *Z. costaricensis* presents expansions of BT1 and amino acids transporter genes. We have also confirmed the evolutionary relationships of both species within the subfamily Leishmaniinae by using supermatrix (3,984 protein coding genes) and supertree methods. Overall, this study showed new expansions of multigene families into monoxenous and dixenous parasites of the Leishmaniinae subfamily. **Keywords:**genomics;Trypanosomatidae;genome evolution.

RT.06-02 - **VEuPathDB: Omics support for the global parasite, vector and fungal research communities**

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VEuPathDB (https://veupathdb.org), is a family of free, online bioinformatics resources supporting >500 species including protozoan parasites, fungi and oomycetes, arthropod vectors and selected host species. VEuPathDB resources facilitate the discovery of meaningful biological relationships from large volumes of data by integrating pre-analyzed Omics data with advanced search capabilities, data visualization and analysis tools. Specialized analytical or computational skills are not required since tools are offered in a friendly web-interface and supported by an email help desk, video tutorials, webinars, and social media. Available data types include genome sequence and population-level variation data; manually-curated and automatically generated annotation; epigenetic, transcriptomic and proteomic data; and pathway information. In addition, geospatially resolved vector surveillance data is available in the VectorBase tool called MapVEu. To take advantage of data from other species, a phylogenetic framework facilitates cross-species functional inference via orthology. In addition, a Galaxy interface provides a bioinformatics platform for privately analyzing your own large scale data and porting your results to VEuPathDB for comparisons with public data. VEuPathDB's comprehensive data mining resources offer valuable in silico hypothesis development and testing, so that end users can answer questions concerning expression levels and timing, domain presence, gene model integrity and genetic variation before venturing into the laboratory. VEuPathDB is one of two NIAID-supported Bioinformatics Resource Centers and receives additional support from the Wellcome Trust. Please email help@veupathdb.org with questions or suggestions.
Keywords: Bioinformatics;Databases;Genomics.

RT.06-03 - **Mosaic aneuploidy in Leishmania and its role in adaptation**

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Aneuploidy, an imbalance in the copy number of chromosomes in a cell, is generally considered harmful, but in some microorganisms, it can act as an adaptive mechanism against environmental stresses. Leishmania emerged as a new model for aneuploidy studies as it has a plastic genome characterized by rapid and dynamic modulation of chromosomes copy number, which leads to different karyotypes co-existing even among sister parasites in clonal populations (mosaic aneuploidy). In our lab we use (single cell) genomics and other omics approaches to study the role of (mosaic) aneuploidy in adaptation. By combining genomics, transcriptomics, proteomics and metabolomics of highly aneuploid strains of Leishmania donovani we established that aneuploidy has a strong impact on the transcript- and protein abundance levels of affected chromosomes, ultimately correlating with the degree of observed metabolic differences.

Moreover, an indirect effect of aneuploidy could be observed through changes in the levels of proteins related to protein folding pathways such as chaperones, chaperonins and heat shock proteins. In the second series of experiments, we applied a high throughput single-cell genome sequencing (SCGS) technology to investigate the extent of mosaic aneuploidy in two distinct clonal L. donovani populations, in standard in vitro culture. We revealed for the first time the complete karyotype of thousands of individual Leishmania parasites, identifying hundreds of karyotypes co-existing in each population. Finally, by combining single-cell genomics, lineage tracing with cellular barcodes and longitudinal genome characterization we investigated the dynamics of mosaic aneuploidy during adaptation to high drug pressure (Sb III and miltefosine) in vitro. We found that aneuploidy changes under Sb III pressure result from the polyclonal selection of pre-existing karyotypes, complemented by further and rapid de novo alterations in chromosome copy number along evolution. In the case of miltefosine, early parasite adaptation was associated with independent point mutations in a miltefosine transporter gene and aneuploidy changes only emerged later, upon exposure to increased concentration of the drug. Thus, polyclonality and genome plasticity are hallmarks of parasite adaptation, but the scenario of aneuploidy dynamics is dependent on the nature and strength of the environmental stress as well as on the existence of other pre-adaptive mechanisms.

RT.06-04 - **Updated Genome Assemblies and Metagenomics of Phlebotomus papatasi and Lutzomyia longipalpis**

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Sand flies serve as vectors for several established, emerging and re-emerging infectious agents. As important vectors of human disease, phlebotomine sand flies are of global significance to human health, transmitting protozoan, bacterial, and viral pathogens, the most devastating which is leishmaniasis. Here we present high-quality chromosome-level genome assemblies of both Phlebotomus papatasi and Lutzomyia longipalpis. Single individual males were sequenced by PacBio HiFi using ultralow input library preparation and additional material was used to generate HiC data for scaffolding. The L. longipalpis genome size was estimated to be 148 Mb and assigned to four chromosomes with 53x coverage and no unplaced scaffolds. The genome coverage for P. papatasi was 14.1x with an estimatedgenome size of 352 Mb with five chromosomes and 640 unplaced scaffolds (26 Mb). Additionally, microbiome species were annotated from DNA sequences of individual P. papatasi females from Afghanistan, Tunisia, and Egypt and L. longipalpis males from five different populations in Brazil (Jacobina, Marajo, Sobral 1S, Sobral 2s, Laphina. In these field-collected sand flies, individuals separated into distinct clusters reflecting their collection site. Interestingly, the sympatric L. longipalpis Sobral 1S and Sobral 2S populations were more similar to allopatric populations than each other. This characterization of microbial communities in wild sand fly populations will allow further exploration of how the microbiome can be manipulated to alter vector-parasite survival and transmission efficiency. **Keywords:**Sand flies;Genome;Micro biome.

HP-01 - **Iron and heme status at the Leishmania-host interface: effect of iron deficiency anemia on the Leishmania (L.) amazonensis virulence.**

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Leishmaniases, a spectrum of diseases caused by *Leishmania*, affect millions of people worldwide. During infection, nutrient availability within the phagolysosomes has significant effects on parasite replication and virulence. This process requires the acquisition of essential nutrients such as iron and heme from the host since *Leishmania* lacks iron storage proteins or the capacity to synthesize heme. Importantly, free iron and heme are cytotoxic as they trigger the generation of free radicals in the presence of oxygen. *Leishmania*, therefore, must acquire heme and iron to survive in a hostile environment that restricts the availability of nutrients to the pathogen, a process called nutritional immunity. Identifying the several proteins that participate in the transport of iron and heme was crucial for understanding these metabolic pathways in *Leishmania.* Here, we sought to investigate the cross-regulation between the two membrane transporters, *Leishmania* Iron Transport 1 (LIT1) and *Leishmania* Heme Response 1 (LHR1), and the effect of host iron and heme availability on *L. amazonensis* infection. We generated mutants overexpressing GFP-tagged LIT1 and LHR1 in WT and LHR1 SKO lines. These mutants are characterized regarding intracellular heme levels, promastigote growth profile under normal and heme-depleted conditions, and *in vitro* infectivity. Promastigote growth profile in heme-depleted conditions revealed that LIT1 overexpression may compensate for the loss of one LHR1 allele. Besides, numerous unsuccessful attempts to generate LIT1 KO by CRISPR-Cas9 indicate that, despite previous publications, LIT1 may be essential. At last, preliminary results on the impact of iron deficiency anemia on *L. amazonensis* infection in mice showed that iron-deficient anemic mice developed smaller cutaneous lesions than healthy mice. Such findings could contribute to in-depth knowledge of iron and heme metabolic pathways and unveil the importance of nutritional immunity on leishmaniases. **Supported by:**FAPESP 2021/03355-0 **Keywords:**leishmaniases;host-pathogen interaction;nutritional immunity.

HP-02 - **Functional characterization of the Asparagine Synthetases of *Trypanosoma cruzi***

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During its life cycle, *Trypanosoma cruzi* undergoes morphological and biochemical changes to adapt to considerably different environmental conditions. In this respect, amino acids and their derivatives are part of the toolbox used by the parasite to cope with such conditions and it has been reported their paramount roles in biological processes other than protein biosynthesis, serving as carbon and energy sources, and participating in host cell invasion, differentiation and resistance to different types of stress. Regarding the role of Asn, we have shown its involvement in several cell processes. In this work we explore the functional role of the enzyme that catalyzes the production of Asn in *T. cruzi*. For this, using CRISPR-Cas9 system, we produced partial and total knockout lineages for the gene encoding the Asn Synthetase (TriTrypDB TcCLB.503625.10) and then phenotypically characterized evaluating processes such as proliferation, host cell infection and trypomastigotes liberation, metacyclogenesis, viability, mitochondrial respiration, resistance to oxidative stress and to accumulation of ammonia (NH_4^+). Although no significant differences in the epimastigoytes proliferation, cell viability, mitochondrial respiration and resistance to oxidative stress were observed for the mutant lineages, there were differences in the metacyclogenesis, since we observed an increase of about 30% in the number of trypomastigotes in the partial knockout lineages incubated in TAU+3AAG medium and an increase of about 20% when these parasites were incubated in TAU+Asn when compared to the control Cas 9; we also observed a decrease in the resistance to ammonia accumulation in the partial knockout lineages(IC50<30mM), and interestingly, an increase in the resistance (IC50>30mM) in the total knockout lineages. We intend to perform further evaluations of these lineages, currently we are producing the Add-back lineages along with the biochemical characterization of the recombinant protein. **Supported by:**Fundação de Amparo à Pesquisa do Estado de SP, Processo: 2022/12315-5 **Keywords:**Trypanosoma cruzi;Asparagine Synthetase;ammonia.

HP-03 - **First report of Leishmania RNA virus 2 (LRV2) in Leishmania infantum strains from canine and human visceral leishmaniasis in the Southeast of Brazil**

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Protistan parasites of the genus *Leishmania* (Kinetoplastea: Trypanosomatidae) are causative agents of leishmaniasis, one of the most important vector-borne diseases prevalent in almost 100 countries. This disease is known to present a broad range of clinical manifestations, a result of multiple interactions between the parasite and the host immune system. However, despite the substantial efforts to unravel the molecular mechanisms governing this interactive interface, they are still not fully understood. A presence of endosymbiotic *Leishmania* viruses has been suggested as an important piece in this puzzle. *Leishmania RNA virus 1* (LRV1) in South American *Leishmania* are commonly found in parasites belonging to the subgenus *Viannia*, while *Leishmania RNA virus 2* (LRV2) were thought to be restricted to the Old-World pathogens of the subgenus *Leishmania*. In this work, we searched for LRV2 in 71 strains of *Leishmania* (*L.*) *infantum*, the causative agent of visceral leishmaniasis (VL), from distinct hosts, clinical forms, and geographical origins. Seventy-one isolates were examined for LRV2. RNAs were isolated from 1 x 10^8 cells using the Trizol™ (Invitrogen) method following the manufacturer's guidelines (Life Technologies/Thermo Fisher Scientific, Carlsbad, CA). One hundred ng of each RNA sample was treated with DNAse I prior to complementary DNA (cDNA) synthesis. Reverse transcription was performed using the Super Script III- First Strand Synthesis Kit, with random hexamer primers following the manufacturer's specifications and LRV1/2 specific primers. Two strains, one isolated from the canine (CUR268) and from human (HP-EMO) cases in the southeastern of Brazil were positive for LRV2. To the best of our knowledge, this is the first detection of LRV2 in New World. **Supported by:**CNPq - 302972/2019-6
Keywords: Leishmania infantum;LRV2;endosymbionts.

HP-04 - **Phase I and II of NasoLeish®, a novel vaccine against canine visceral leishmaniasis caused by *Leishmania infantum.***

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Visceral leishmaniasis is a lethal neglected disease caused by *Leishmania infantum* and *L. donovani*. In Brazil, dogs are the main reservoir of *L. infantum*. We designed a novel vaccine formulation, NasoLeish®, for intranasal administration to newborn puppies consisting of *L. amazonensis* antigens associated with retinoic acid nanostructured with pegylated liposomes as an adjuvant. Our pre-clinical tests in mice demonstrated the efficacy of intranasal NasoLeish® in preventing parasite growth in the spleen, liver and bone marrow after infection. Also, a clinical trial with 23 *L. infantum*-free mongrel dogs, non-reactive by SNAP, ELISA and PCR, was concluded in 2022. Dogs received two intranasal doses of NasoLeish® at 15- day intervals, or 3 subcutaneous doses of the marketed vaccine Leish-Tec® as controls. NasoLeish® showed to be immunogenic, increasing the serum levels of anti-promastigote IgG, IgA and IgM and anti-amastigote IgG. Of note, all dogs tested negative for the DPP rapid test 30 days after the last dose, indicative of vaccine non-interference with current CVL diagnostic test. We also demonstrated that the vaccine leads to an increased production of IFN-γ and TGF-β while reduces the production of IL-10. Dogs were challenged with *L. infantum* 3 months after vaccination and tested for PCR 2 months after infection. We could see that NasoLeish® strongly reduce the parasite burden at spleen, liver and bone marrow. Overall, NasoLeish® is a promising candidate for the prevention of canine visceral leishmaniasis caused by *L. infantum*. **Supported by:**FAPERJ, GCRF **Keywords:**Canine visceral leishmaniasis;Intranasal vaccine;Mucosa.

PV-01 - **Role of α-tubulin acetylation in the cell cycle of *Trypanosoma cruzi***

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The most abundant isoform in all microtubular structures of Trypanosomatids is acetylated α-tubulin. Acetylation on K40 of α-tubulin is conserved from lower eukaryotes to mammals and is associated with microtubule stability. It is also known that it occurs significantly on flagella, centrioles, cilia, basal body and the mitotic spindle. The primary acetyltransferase that delivers this modification was recently identified as Mec-17/ATAT, a Gcn5-related N-acetyltransferase. Despite evidence supporting a role for K40 acetylation in microtubule stability, its biological function *in vivo* remains unclear. To study α-tubulin K40 acetylation we employed different genetic manipulation strategies in *Trypanosoma cruzi*. We analyzed the phenotypes of the resulting parasites using expansion, confocal and electron microscopy. Firstly we have expressed *Tc*ATAT in epimastigotes using the inducible vector pTcINDEX-GW. *Tc*ATAT is located in the cytoskeleton and flagella, colocalizes with acetylated α-tubulin in these structures and over-expression causes increased levels of the acetylated isoform and a halt in the cell cycle progression of epimastigotes. Also, these parasites become more resistant to microtubule depolymerizing drugs. Then we used the same system to over-express mutant versions of α-tubulin K40, which generates reduced levels of acetylated α-tubulin, quantified by flow cytometry. Also, the cell cycle progression is altered and an aberrant morphology is observed in these parasites. Finally we obtained *Tc*ATAT knock-out epimastigotes by CRISPR/Cas9, these parasite have undetectable levels of acetylated α-tubulin and severe defects in there replication rates, their motility is impaired and present a detached flagella. These evidence supports the idea that α-tubulin acetylation is crucial for *T. cruzi* replication and cell cycle progression and that *Tc*ATAT is responsible for this posttranslational modification. **Supported by:**Agencia Nacional de Promoción Cientifica y Tecnológica, Argentina (PICT2019-0526, PICT2021-0157), CONICET (PIBAA 1242) **Keywords:**Cytoskeleton;Acetylation;Tubulin.

PV-02 - **Development of paratransgenic *Lutzomyia longipalpis* by engineered bacteria driving refractoriness to *Leishmania infantum chagasi* infection.**

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The parasite *Leishmania infantum chagasi* is the etiologic agent of American visceral leishmaniasis (AVL), a disease transmitted by the sandfly *Lutzomyia longipalpis.* The use of insecticides to control vector populations is one of the main strategies to limit the spread of AVL. However, this approach has limitations, including drug resistance and growing socioecological impact due to the need for more potent compounds. The current scenario of territorial expansion with increasing incidence and lethality of AVL highlights the need for new strategies to control the spread of this disease. Paratransgenesis is a approach in which symbiotic bacteria of insect vectors are engineered to drive antiparasitic effector molecules and reduce their vectorial capacity. To find candidate bacteria for genetic manipulation, we isolated culturable bacteria that were enriched in the gut of *L. longipalpis* after blood feeding. We are identifying these symbionts through 16S rRNA sequencing and working to establish a genetically engineered lineage. We determined that 16μM of the antimicrobial peptide (AMP) Melittin was toxic to *L. infantum chagasi* axenic cultures and in artificially infected *L. longipalpis* females, but had no deleterious effect on the vector or bacterial symbionts isolated from their gut. Now we are testing the combination of other AMPs with Melittin to observe the occurrence of a synergistic anti-leishmanial activity. We are genetically engineering *Escherichia coli* strains C600 and BL21 with a plasmid expression vector encoding the PhoA signal peptide to express and secrete the AMP Melittin constitutively. This plasmid stability and Melittin secretion are being assayed in *E. coli.* Our next steps include testing the effect of transgenic *E. coli* conditioned media on *L. infantum chagasi* cultures and during their development in *L. longipalpis.* In this way, we propose the development of a paratransgenic sandfly with reduced vetorial capacity to transmit the *L. infantum chagasi.* **Supported by:**CAPES **Keywords:**Lutzomyia longipalpis;Leishmania infantum chagasi;Paratransgenesis.

PV-03 - **The three-dimensional genome organization in *Trypanosoma cruzi***

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In *Trypanosoma cruzi*, gene expression is broadly determined by post-transcriptional mechanisms. However, a significant decrease in global transcription has been observed to distinct life forms, suggesting some layer of transcription regulation (Elias *et al.,* 2001). The genome of *T. cruzi* is compartmentalized: conserved genes form the core compartment, and the multigenic family of genes encoding surface proteins composes the disruptive compartment remarked by synteny break (Berná *et al.,* 2018). Previously, we found core and disruptive genes preferentially located at open and closed chromatin regions, respectively (Lima *et al.,* 2020). The present work aims to understand how different genomic features are organized into 3D genomic structures and to what extent the nuclear architecture influences gene expression. We evaluated the *T. cruzi* genome-wide chromosome conformation capture (Hi-C) public data (Tarleton, *et al.,* 2018) against the following set of genomic features: compartments, small RNAs, transcription start and end sites, pseudogenes and DNA repeats. We characterized core and disruptive rich Topological Associated Domains-like structures (TAD-like), in which disruptive rich TAD-like domains cover shorter genomic regions. We detected tDNAs, pseudogenes and transcription termination sites preferentially located at the boundary of TAD-like domains. Currently, we are investigating the enrichment of genomic features at TAD boundaries in between i. two open-open or closed-closed chromatin regions; or ii. in between two open-closed chromatin regions. This 3D genome organization suggests a range of chromatin folding patterns and opens a new question that we aim to address: "is gene expression impacted by the positioning of genomic features at the boundaries of TAD-like domains?" **Supported by:**FAPESP 2021/03219-0 **Keywords:**genome-wide chromosome conformation capture;3D genome organization;T; cruzi.

PV-04 - **Iron Uptake Controls *Trypanosoma cruzi* Metabolic Shift and Cell Proliferation**

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(1) Background: Ionic transport in *Trypanosoma cruzi* is the object of intense studies. *T. cruzi* expresses a Fe-reductase (TcFR) and a Fe transporter (TcIT). We investigated the effect of Fe depletion and Fe supplementation on different structures and functions of *T. cruzi* epimastigotes in culture. (2) Methods: We investigated growth and metacyclogenesis, variations of intracellular Fe, endocytosis of transferrin, hemoglobin, and albumin by cell cytometry, structural changes of organelles by transmission electron microscopy, O_2 consumption by oximetry, mitochondrial membrane potential measuring JC-1 fluorescence at different wavelengths, intracellular ATP by bioluminescence, succinate-cytochrome c oxidoreductase following reduction of ferricytochrome c, production of H_2O_2 following oxidation of the Amplex® red probe, superoxide dismutase (SOD) activity following the reduction of nitroblue tetrazolium, expression of SOD, elements of the protein kinase A (PKA) signaling, TcFR and TcIT by quantitative PCR, PKA activity by luminescence, glyceraldehyde-3-phosphate dehydrogenase abundance and activity by Western blotting and NAD^+ reduction, and glucokinase activity recording $NADP^+$ reduction. (3) Results: Fe depletion increased oxidative stress, inhibited mitochondrial function and ATP formation, increased lipid accumulation in the reservosomes, and inhibited differentiation toward trypomastigotes, with the simultaneous metabolic shift from respiration to glycolysis. (4) Conclusion: The processes modulated for ionic Fe provide energy for the *T. cruzi* life cycle and the propagation of Chagas disease. **Supported by:**FAPERJ, E-26/203.901/2021 **Keywords:**ATP synthesis;mitochondrial function;parasite oxidative stress.

PV-05 - **Mitochondrial inheritance in *Toxoplasma gondii* is dependent on actin and myosin A**

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Toxoplasma gondii's single mitochondrion is highly dynamic and changes morphology when the parasite is exposed to different environments. This parasite divides by endodyogeny, where two daughter cells are formed inside of a mother cell. The organellar division is tightly regulated with the cell cycle, and the mitochondrion is the last organelle to be divided. Parasite's mitochondrial dynamic is dependent on a protein named **L**asso **M**aintenance **F**actor **1** (**LMF1**). LMF1 mediates mitochondrial dynamics by interacting with IMC10, a protein localized at the inner membrane complex (IMC). LMF1 and IMC10 form a unique tether between the mitochondrion and the IMC. As little is known about mitochondrial inheritance, we have used the LMF1/IMC10 interaction as an entry point to dissect the machinery behind this process. Using a yeast two-hybrid screen for putative LMF1 interactions, we identified Myosin A (MyoA) as a strong candidate. Although MyoA is known to be located at the parasite's IMC, ultrastructure expansion microscopy (U-ExM) shows that this protein accumulates around the mitochondrion in the late stages of division. Parasites lacking MyoA show defective mitochondrial morphology, and a delay in mitochondrion delivery to the buds during division, indicating that this protein is involved in organellar inheritance. Disruption of the parasite's actin network with Cytochalasin D also affects mitochondrion morphology. We have shown that parasite-extracted mitochondrion vesicles interact with actin filaments. Interestingly, mitochondrion vesicles extracted out of parasites lacking LMF1 pulled down less actin, showing that LMF1 might be important for mitochondrion and actin interaction. Accordingly, we are showing for the first time that actin and Myosin A are important for Toxoplasma mitochondrial inheritance. Current work includes characterizing other proteins involved in mitochondrial inheritance and how MyoA affects organellar speed during mitochondrion segregation. **Supported by:**NIH grants R01AI123457, R01AI149766, R01AI89808, and R21AI124067 to G.A. and in part, with support from the Indiana CTSI funded, in part by Grant Number UL1TR002529 from the NIH, NCATS, Clinical and Translational Sciences Award to R.O.O.S. **Keywords:**Mitochondrion;Actin;Myosin A.

PV-06 - **ACCESSING THE GENOMIC ELEMENTS AND STRUCTURAL VARIANTS BETWEEN *Crithidia sp* LVH60a AND *C. fasciculata* GENOMES**

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An increase in occurrence of monoxenic trypanosomatids, such as *Crithidia* and *Leptomona*s, has been observed in human cases of leishmaniasis. We have shown that some clinical isolates from VL (visceral leishmaniasis) cases in Sergipe, Brazil, do not belong to the *Leishmania* genus and are phylogenetically related to *Crithidia*, a monoxenous trypanosomatid considered non-pathogenic to humans. In order to characterize specific genomic elements and structural variations between *Crithidia sp* LVH60a-C1 strain and *C. fasciculata* reference, we performed whole-genome pairwise alignment analysis using MUMmer to calculate the average nucleotide identity (ANI) and Artemis to observe pairwise comparisons between the genomes of these two trypanosomatids. To validate the assembly chromosome features, we performed polymerase chain reactions utilizing LVH60a-C1 and TCC039E (*C. fasciculata*) strains. The alignment of LVH60a-C1 and *C. fasciculata* genomes exhibited an ANI ranging from 92.7% to 94.8%. We also observed chromosomal rearrangements of LVH60a-C1 when compared to *C. fasciculata* CfCl genome. We are validating arrangements in LVH60a-C1 genome by PCR regarding chromosomal positions of *C. fasciculata* as reference. So far, we validated two structural genomic arrangements in putative *Crithidia* LVH60a-C1 chromosomes: 1) Contig 25: 0.90 Mb syntenic to *C. fasciculata* chromosome 2 (0.53 Mb) fusioned with a 0.30 Mb fragment of *C. fasciculata* chromosome 29 (2.19 Mb); 2) Contig 12: partial duplication and inversion (0.04 Kb) of *C. fasciculata* chromosome 8. It is important to note that there is no established ANI threshold to classify trypanosomatids within the same species, however, previous studies have reported ANI values of around 94% between dermotropic and viscerotropic species of *Leishmania*. Our results may indicate a potential novel species within *Crithidia* genus, but more isolates need to be analyzed with focus on populational genomics to ascertain this evidence. **Supported by:**São Paulo Research Foundation (FAPESP) grant 2016/20258-0 and 2020/14011-8; CAPES; CNPq **Keywords:**Crithidia sp;;comparative genome;chromosomal rearrangements.

PV-07 - **Loss of RNase H1 in *Leishmania* leads to an altered DNA replication programme and increased genome variability.**

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Genome plasticity, manifested as gene and chromosome copy number variation, is an effective survival strategy used by *Leishmania* parasites and therefore stands as one of the stumbling blocks for developing effective anti-leishmanial therapies. However, our understanding about the mechanisms underlying genome malleability in this parasite remains limited. Here, we set out to investigate if and how R-loops contribute to genome variability in *Leishmania*. By using DRIP-seq, we show that a major locus for R-loop localization is at sites of coupled trans-splicing and polyadenylation, needed to generate mature mRNA. Globally, however, R-loop distribution within each *L. major* chromosome parallels the spatial and temporal compartments of the parasite's unconventional DNA replication programme. Strikingly, R-loop levels correlate with chromosome size, which in turn correlate with replication timing. In addition, R-loop levels are lower in subtelomeres, where replication can occur outside S-phase. DiCre-mediated inducible *KO* of *RNase H1* reveals a transient and dramatic growth perturbation, with resulting null mutants displaying pronounced changes in gene expression as revealed by RNA-seq. By performing MFA-seq analysis, we also observed dramatic changes in the DNA replication programme upon *RNAse H1* deletion. We also performed whole genome sequencing and observed increased levels of genome-wide, chromosome-size dependent instability, including aneuploidy, SNPs and InDels upon *RNAse H1 KO*. Finally, ChIP-seq analyses shows that *L. major* RNAse H1 is enriched at origins of DNA replication and, to a lesser extent, at transcription boundaries. We hypothesise our data reveal a crucial role for *Leishmania* RNase H1 in controlling the DNA replication programme by maintaining proper levels of R-loop and place the DNA-RNA hybrids as a pivotal player in *Leishmania* genome plasticity. **Supported by:**Wellcome, MRC, European Commission-Marie Skłodowska-Curie Actions **Keywords:**DNA Replication and Repair;R-loops and RNAse H1;Genome Plasticity.

PV-08 - **Diversification of Heterotrophic Microeukaryotes: Insights from Arcellinida and Related Amoebozoan Taxa**

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Heterotrophic microbial eukaryotes play a pivotal role in marine and terrestrial ecosystems, contributing to carbon and nutrient cycles. These microorganisms, capable of phagocytosis, act as predators on bacterial communities and other microeukaryotes, occupying a significant position in complex food webs. The origin and diversification of these heterotrophic microeukaryotes remain unclear. Fossil evidence and molecular data suggest that the emergence of predatory microeukaryotes and the transition to a eukaryote-dominant marine environment occurred around 800 million years ago. Vase-shaped microfossils (VSMs) represent the oldest known evidence of heterotrophic microeukaryotes in marine environments and terrestrial habitats. In this study, we investigate the early divergence and diversification of Arcellinida and related amoebozoan taxa using a relaxed molecular clock approach. Our phylogenomic analysis reveals a well-resolved tree of amoebozoan testate amoebae, including a monophyletic Arcellinida with three suborders and five infraorders. Through calibration using fossils and rigorous clock models, we estimate the timing of diversification of Arcellinida during the early Neoproterozoic, shedding light on the expansion of life during this period. Our results suggest a well-established complexity in shallow marine ecosystems, involving both phototrophic and heterotrophic microeukaryotes during the neoproterozoic, followed by an invasion of freshwater systems and subsequent diversification of Arcellinida in the Phanerozoic. The findings indicate the need for some revision of the classification of amoebozoan testate amoebae, with taxa reassigned to different orders. Overall, this study provides valuable insights into the evolutionary history and ecological significance of heterotrophic microeukaryotes in Earth's ecosystems. **Supported by:**FAPESP 19/22815-2 **Keywords:**Arcellinida;Phylogenomic;Fossils.

PV-09 - **Unveiling a novel Isoprenoid Salvage Pathway in *Plasmodium falciparum*: new perspectives in fosmidomycin as an antimalarial**

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Malaria, predominantly caused by the *Plasmodium falciparum* pathogen, remains an endemic problem in tropical and subtropical regions. The emergence of drug resistance is a major problem, highlighting the need for new antimalarial compounds. Fosmidomycin (FOS) inhibits the methylerythritol 4-phosphate (MEP) pathway, crucial for isoprene unit biosynthesis. Despite its efficacy, recurrent cases of recrudescence have led to investigations into the biological causes of FOS recovery. This study found isoprenoid alcohols such as farnesol (FOH), geranylgeraniol (GGOH), phytol (POH), and unsaponifiable lipid extracts from foods can restore FOS activity in *P. falciparum*. These substances are phosphorylated, lengthened, and incorporated into proteins. Proteomic and radiolabeling studies showed that prenylated proteins can bind to several isoprenoids if externally supplied. A gene (Pfpolk) encoding a prenol kinase was identified, which when expressed in yeast, exhibited farnesol and geranylgeraniol kinase activities. Conditional knockout parasites (ΔPfpolk) were created using CRISPR-Cas9 and DiCre strategies, to investigate the biological importance of the farnesol/geranylgeraniol salvage pathway. ΔPfpolk parasites were more susceptible to MEP inhibitors and incapable of using isoprenoid alcohols for protein prenylation. The study suggests that the farnesol and geranylgeraniol salvage pathway is an additional isoprenoid source for malaria parasites. Inhibition of this pathway could enhance the effectiveness of drugs targeting isoprenoid metabolism. A compound reducing the FOS-recovering effect of geranylgeraniol was identified, making it a potential candidate for co-use with FOS in trials. Collectively, these findings deepen our understanding of the action mechanisms of antimalarials targeting the apicoplast and shed light on a novel post-translational modification of proteins in *P. falciparum*, providing valuable insights for the development of more effective antimalarial drugs.
Supported by:FAPESP, 2018/02924-9 e 2018/02924-9 **Keywords:**Malaria;Isoprenoid;Drug.

PV-10 - **Molecular Insights into the Evolutionary Origin and Mechanisms of Shell Formation in Amoebozoan Testate Amoebae (Arcellinida:Amoebozoa)**

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The emergence of evolutionary novelties is a central question in evolutionary biology. Some amoeboid organisms have an outer covering (shell), and this feature is an evolutionary novelty in the group of Arcellinida testate amoebae. While arcellinid's shell formation process has been well documented in the literature based on morphological data, we currently lack an understanding of the molecular apparatus underlying this process, impairing the inference of the evolutionary events involved in its origin. Here we report a hypothetical model of the molecular pathway for the shell formation process derived from morphological descriptions and supported by de novo sequencing, assembly, and annotation of single-cell transcriptomes of Arcella intermedia. Our model suggests that the origin of the shell formation process involved the co-option, with gene duplication events, of the molecular machinery that controls cell polarization and regulative exocytosis in eukaryotes. Further investigation based on immunofluorescence and other methods will enable us to test the model in situ and describe the shell formation process molecularly. Ultimately, we aim to gain insights into the origin and evolution of the shell formation process in Arcellinida. **Supported by:**FAPESP - 2019/22692-8 **Keywords:**Evolutionary Cell Biology;Omics;Differential gene expression.

PV-11 - **Emerging-3'UTR ncRNAs of duplicated genes in *Leishmania major*: what is their relevance?**

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Noncoding RNAs (ncRNAs) are transcripts with low translational potential that play a role in many different human diseases. Here, two ncRNAs (c12 and OOD3) emerging from the 3'UTR of duplicated genes in *Leishmania major* were investigated by multi-colour single molecule fluorescence *in situ* hybridisation (smFISH) in order to prove their presence and characterise location, expression levels, stability and biogenesis. For both predicted ncRNAs, smFISH detected about similar ratio of the ncRNA signal as free and presented within the 3´UTR of the cognate mRNA. ncRNA_c12 was preferentially cytoplasmic (~80%) while a similar distribution between nucleus and cytoplasm was found for the ODD3. Both ncRNAs were less stable than the coding genes they were derived from and, when found as 3'UTR constituent, did not affect the stability of their cognate transcripts. The levels of free ncRNAs in the cell decreased by blocking the trans-splicing for 30min, which can also be correlated to their short stability. However, the level of c12 co-localised with the cognate gene was not affect by trans-splicing inhibition, even at 2h post sinefungin treatment, directly related to its stability. By treating the total RNA with different RNA modification enzymes, we confirmed the presence of CAP at 5' end of ODD3 transcript. However, c12 was identified as partially capped, maybe related to the intracellular location of this ncRNA. Lacking or overexpressor parasites for ODD3 lead to consistent changes in the transcriptome, indicating possibly its action as a trans-regulatory RNA. All the evidence aggregates relevant and new insights about the function of these 3'UTR emerging ncRNAs, considered as mRNA sub-product so far. Interestingly, despite similar genomic origin, ODD3 and c12 have different biogenesis and processing. In some way, the parasite has peculiar and still unearthed machinery(ies) responsible to distinguish similar ncRNAs and give function for some of them. **Supported by:**FAPESP 2020/00088-9 / 2021- **Keywords:**noncoding RNA;RNA-FISH;Leishmania.

PV-12 - **Recombinant Expression of *Trypanosoma cruzi* Histones for Nucleosomes Assembly**

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Chagas Disease, caused by the parasite ***Trypanosoma cruzi***, continues to afflict millions of individuals worldwide, lacking effective vaccines and proper treatment options for its chronic phase. The infection and survival of the parasite within the host involves the regulation of gene expression at the post-transcriptional level. Emerging evidence suggests that epigenetic mechanisms play a pivotal role in the biology of these parasites, presenting a promising approach for targeted drug discovery. To unravel the complex workings of these mechanisms, it becomes imperative to explore the chromatin organization, the atomic structure of histones and, therefore, the nucleosome core particles (NCPs). This study aims to undertake a comprehensive investigation into the structure of histones, produced through recombinant expression techniques, as well as the NCPs themselves, using Cryogenic Electron Microscopy (Cryo-EM) techniques. Moreover, it seeks to investigate the kinetic and thermodynamic properties governing NCP assembly and its interaction with DNA. To achieve these objectives, both canonical histones (H2A, H2B, H3, and H4) and variant histones (H2A.Z, H2B.V, H3.V, and H4.V) are being expressed with the aid of single-gene as well as polycistronic constructs to overcome instabilities in the natural folding of proteins. The biophysical aspects of NCP formation and its interaction with specific regions of ***T. cruzi***'s genome are being evaluated using advanced fluorescence anisotropy techniques. Structural investigations will be conducted using Cryo-EM in order to attain a comprehensive understanding of the organization and function of NCPs in ***T. cruzi***. The preliminary results obtained thus far underscore the critical importance of further studies to acquire the necessary knowledge regarding the genetic regulation of ***T. cruzi***, thereby laying the foundation for the development of innovative and effective drugs. **Supported by:**Agência financiadora: CNPq; Nº do processo: 130990/2022-1 **Keywords:**Cryogenic Electron Microscopy;Nucleosome Core Particle;Trypanosoma cruzi.

TB-01 - **RNA BINDING PROTEINS AS TRANS-REGULATORS IMPACTING SURVEILLANCE AND INFECTIVITY IN *LEISHMANIA.***

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Leishmania spp. protozoan Kinetoplastids present peculiar gene expression fundamentally dependent upon post-transcriptional control. This elevates the importance of RNA binding proteins for gene regulation in these parasites. Building upon the mRBPome we isolated previously (Pablos, Ferreira et al., MCP, 2019), 70 mRNA-bound RBPs were selected from the three main *L.mexicana* lifecycle stages. A trans-regulator knockout clone library was created through barcoded CRISPR and screened for essential roles in cellular differentiation and macrophage or mouse infections. Of the 70 RBPs screened, 40 are essential candidates to cell viability and 18 contribute to lifecycle progression to human-infective stages and/or parasite infectivity. Examination of individual knockout lines for amastigote-specific mRBPs showed normal promastigote growth dynamics, whereas macrophage infection was inhibited or ablated, suggesting essential roles of RBPs for amastigote viability and virulence. Nine mRBPs were Immunoprecipitated and submitted to transcriptomic analysis for to identify associated transcript targets that may represent novel virulence factors. **Supported by:**MRC, GCRF, NTD **Keywords:**LEISHMANIA;RNA Binding Protein;INFECTION.

TB-02 - **DEVELOPMENT OF AMPHOTERICIN B MICROPARTICULATED IMPLANTS BY SPRAY-DRYING FOR LOCAL TREATMENT OF CUTANEOUS LEISHMANIASIS**

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Localized cutaneous leishmaniasis (LCL) is the most common form of the disease. Intralesional injections with Glucantime antimonial have reduced the systemic toxicity produced by parenteral injections, but they are painful, often require repetition, and are only applicable in medical posts. We have envisaged to develop novel drug formulations to allow a single local injection to be effective in LCL. Previously, we showed the promising use of biodegradable PLGA (poly(lactide-co-glycolide acid) microparticles (Mcps) loaded with amphotericin B (AmB) for sustained drug release. Here, we proposed to improve the process of AmB/PLGA Mcps production by using the spray-drying instead of the previous emulsification-solvent evaporation method. Spray-drying allows scale-up production and the use of less toxic solvents and surfactants. Using an appliance equipped with a three-fluid nozzle set, we produced Mcps containing AmB in a shell-core design. Fabrication process showed 60-70% yield; average particle size of 13.5 µm; and >70% drug incorporation rate. MEV images showed spherical and rough particle topology. *In vitro* drug release kinetics showed that AmB/PLGA Mcps promoted much slower release than free AmB. DSC and FTIR analyses showed that the spray-drying process did not change AmB and PLGA physical and -chemical characteristics. Murine macrophages exposed to AmB/PLGA Mcps did not show signs of toxicity. Mice injected s.c. with AmB/PLGA showed a transient local inflammation as monitored by MEST and histopathology. BALB/c mice infected in the ear pina with *Leishmania amazonensis*-GFP and given a single s.c. injection with AmB/PLGA showed significantly smaller lesion growth and parasite burden as compared with free AmB (Anforicin B®). This study shows that the green spray-drying method can produce industrially feasible PLGA/AmB Mcp implants for an effective treatment of LCL with a single injection with AmB, and possibly with other newly discovered antileishmanial drugs. **Supported by:**CAPES nº 88887.374118/2019-00 e VALE S.A. **Keywords:**Cutaneous leishmaniasis;Amphotericin b;Spray-drying.

TB-03 - **A genome-wide yeast surface display screen to identify vaccine targets for Chagas disease**

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Chagas disease (ChD) affects roughly 8 million people and is caused by infection with the protozoan parasite *Trypanosoma cruzi.* The resulting pathology begins with an acute infection presenting mild flu-like symptoms and then progresses to the chronic stage, which can be lethal. There are no vaccines available against ChD, and the existing drugs – nifurtimox and benznidazole – are highly toxic with limited efficacy in the chronic stage. We developed a *T. cruzi* genome-wide library for yeast surface display (YSD) to identify immunogenic proteins for vaccine development. Nanopore sequencing of the library showed a 30-fold coverage of the parasite genome with all parasite genes included and library fragments ranging between 0.5 to 3 kb. Computational prediction of the *T. cruzi* library-expressed proteins in the YSD system based on nanopore sequencing showed over 248,946 unique polypeptides. We confirmed successful yeast surface protein expression by flow cytometry and Western blotting. To identify immunogenic polypeptides, we performed five rounds of Magnetic Activated Cell Sorting (MACS) against sera of chronic ChD or healthy patients. About 40% of the library was enriched with sera of chronic ChD patients, and nanopore sequencing of libraries identified a set of ~6,600 ChD-specific immunogenic polypeptides ($p<0.05$; fold change > 2) at nucleotide resolution. The dataset revealed immunogenic peptides of proteins that react specifically to ChD antibodies and those cross-reacting with sera of healthy individuals. The libraries are useful resources for screening drug targets, protein ligands, vaccine targets, and biomarkers. The identified immunogenic antigens are being used for mice mRNA and recombinant protein vaccination studies. **Supported by:**International Development Research Centre (IDRC, 109929-001 to I.C.); Canada Foundation for Innovation (CFI, JELF 258389 to I.C.) **Keywords:**Chagas disease;vaccines;genome-wide yeast surface display screen.

TB-04 - **Whole genome analysis of paromomycin resistant lines and susceptible isolates of *Leishmania amazonensis* reveals changes in chromosomal somy and variable polymorphisms**

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Leishmania amazonensis is the second most prevalent species that causes tegumentary leishmaniasis (TL) in Brazil. The available treatment is limited to the use of few drugs, that are mostly toxic and induce several side effects, in addition to the high-cost treatment. Paromomycin (PM) is a low-cost aminoglycoside antibiotic currently used in the chemotherapy of visceral leishmaniasis in Southeast Asia. Due to the potential of PM against *L. amazonensis* demonstrated in *in vitro* and *in vivo* studies (Coser et al., 2020; Coser et al., 2021) and the limitations of the current treatment, our aim is to identify potential genes associated with PM resistance and susceptibility in this species, using PM-resistant lines selected *in vitro* and two clinical isolates highly susceptible to PM. We sequenced *de novo* the genome of *L. amazonensis* reference strain (M2269), whose sequence is not completely assembled. The whole genome sequence of this strain was obtained through assembling of reads obtained from Nanopore and Illumina platforms and used as reference for investigation of short-nucleotide variants (SNVs), including single and multiple nucleotide polymorphisms and insertions/deletions in coding sequences, and chromosomal ploidy of PM-resistant lines and isolates that were sequenced using Illumina platform. Bioinformatic analyses showed differences in chromosomal ploidy in PM-resistant lines selected through stepwise selection and PM-susceptible isolates compared to the parental M2269, while lines selected by *in vitro* mutagenesis did not show significant changes in ploidy. Preliminary results indicated an average of 800 SNVs between PM-resistant lines and 9,500 SNVs among isolates. This study will contribute for the identification of genes associated with resistance and susceptibility to PM that can be useful to understand the molecular basis of the mechanism of action and resistance in *Leishmania*. **Supported by:**Processos FAPESP 2016/21171-6 e 2019/22175-3; Processo CNPq 405235/2021-6 **Keywords:**Drug resistance;Paromomycin;Whole genome sequencing.

TB-05 - **Paving the road for genomic surveillance of leishmaniasis: source tracing of *L. donovani* in recent outbreaks of visceral leishmaniasis in West Nepal**

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Molecular surveillance of parasitic diseases may provide information of highest relevance for control programs, such as (i) following the evolution of epidemics in time and space, (ii) characterization of new transmission cycles, (iii) outbreak studies and source identification and (iv) detection of new variants with new clinical features. Currently, no molecular surveillance exists for leishmaniasis, despite the existence of suitable technologies. We previously showed the feasibility and significant added value of direct parasite whole genome sequencing (SureSelect-sequencing, SuSL) in host or vector. We further optimized SuSL for 3 different parasite species and different types of clinical samples. Here, we demonstrate the proof-of-principle of SuSL for genome surveillance, in the context of a recent outbreak of visceral leishmaniasis in Western Nepal. Blood samples were collected in 2019 and stored on DNA/RNA Shield. Three samples with highest amounts of DNA, positive for *Leishmania* and from different districts were sequenced, with a high genome coverage. The 3 genomes were compared to our database of *L. donovani* genome sequences in the Indian sub-continent: this revealed that 'outbreak parasites' were distinct from the 'core' population observed between 2002 and 2011 in the lowlands of Nepal, India and Bangladesh. One sample branched close to ISC1, a small population of parasites found more frequently in Nepalese highlands and likely functionally different from the 'core' parasites. Two samples clustered together with a divergent genome previously reported only once. We looked for possible signatures of drug resistance and found several missense mutations in known drug transporters (AQPs and LdMT). Altogether, our results support the need for further genomic surveillance, in particular in the context of the current elimination program in the Indian subcontinent and demonstrate the applicability of SuSL to molecular surveillance of blood. **Supported by:**Belgian Development Cooperation ; Department of Economy, Science and Innovation (Flanders) ; EU-MSCA-RISE (Leishield) **Keywords:**Post-elimination surveillance;Indian sub continent;Genome capture.

TB-06 - **Antileishmanial of Drugs Identified by High Content Screening against Intracellular Amastigotes**

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Leishmaniasis is caused by several species of the protozoan parasites of the genus *Leishmania*. This disease treatment remains difficult, since the available drugs have shown to be highly toxic, besides several effects has been shown. Thus, new therapeutics are urgently needed. High content screening represents an important tool in the drug discovery process, since it optimizes the chances of finding an active compound from a large number of candidates. So, the aim of this work was to evaluate the antileishmanial activity of some commercial drugs by high content screening targeting intracellular amastigotes. Bone marrow-derived macrophages (BMDMs) were plated in 96-well plates, and then infected with *L. amazonensis* expressing RFP. 3h post-infection, cells were treated with the drugs at 10 µM. Then, image acquisition was made 24h, 48h and 72h post-treatment. It was evaluated the antileishmanial activity of 2560 drugs in the initial screening. After that, were selected 80 compounds showing at least 50% of intracellular amastigotes inhibition when compared to controls (treatment with DMSO 1%). After two revalidation assays, 38 still demonstrated antileishmanial activity. Following, these compounds have determined their EC_{50} and CC_{50} values, at this point 26 compounds that demonstrated EC_{50} < 10 and SI > 10 were selected. These 26 selected compounds were repurchased lyophilized and re-tested against intracellular amastigotes, in this step 18 compounds maintained the antileishmanial activity. After other selection steps based on drug approval by regulatory agencies and route of administration, we selected 9 compounds FDA-approved and orally administered to follow in our *in vivo* studies. By the end, was found a drug called as C00 which reduced the ear thickness and parasite burden in ear after 15 days orally administered to mice infected with *L. amazonensis.* **Supported by:**CNPq - 152584/2022-6 **Keywords:**Antileishmanial drug;High content screening;Cutaneous leishmaniasis.

TB-07 - **Search for new Drugs and Potential Molecular Targets Associated with Benznidazole Resistance Phenotype in *Trypanosoma cruzi***

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Chagas disease (CD) is a public health problem in Latin America, caused by *Trypanosoma cruzi*. Nifurtimox and Benznidazole (BZ), the only two available interventions, have low cure rates during the chronic stage of the disease and present several toxic side effects. Recently, we performed a comparative transcriptomic analysis of wild-type and BZ-resistant *T. cruzi* lines, and the results revealed a robust set of genes from different metabolic pathways associated with the BZ-resistant phenotype. In this study, we are identifying new molecular targets and drugs against CD using computational methodologies and drug repositioning strategies looking for those that interact with differentially expressed proteins identified by transcriptomic analysis. The transcriptomic profile of *T. cruzi* revealed a list of 1,819 differentially expressed transcripts. Based on gene expression level and genomic context, we selected one gene for detailed investigation. This gene is unique to the Dm28c strain (TcI) and exhibits similarity to six targets in the DrugBank database. Using CRISPR/Cas9 system, we have successfully deleted one allele of this gene from Dm28c strain, and the deletion of second allele is in process. Among the identified molecular targets, we found 49 drug candidates for replacement, with 25 approved for medical use. The protein-protein interaction network consists of 7,685 proteins and 3,685,439 interactions. We have added node attributes such as resistance data and gene ontologies, and we are developing codes for simulations with network properties. The study of protein-protein interactions enables the identification of new molecular targets relevant to the resistance phenotype, even in the absence of increased expression. Therefore, the integration of *in silico* and *in vitro* approaches in the search for molecular targets and drugs can significantly contribute to the development of new treatments against CD. **Supported by:**Programa INOVA FIOCRUZ – Fundação Oswaldo Cruz (VPPCB-07-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 and RED-00104-22); Convênio UGA/FAPEMIG (APQ-04382 (D)); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 304158/2019-4) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – BRA (CAPES) – Finance code 001. **Keywords:**Drug resistance;Trypanosoma cruzi;Chemotherapy.

HP-05 - **Trans-sialidase as a virulent factor of *Trypanosoma cruzi* involved with parasite egress and the production of pro-inflamatory cytokines by the infected cell**

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Trans-sialidases (TS) are encoded by the largest gene family in the *Typanosoma cruz*i genome in which 12 sequences encode enzymatically active TS (aTS). aTS are responsible for transferring sialic acid from host glycoconjugates to mucins present on the parasite surface. Using CRISPR/Cas9 technology, we generated aTS knockout parasites that display undetectable levels of TS activity. Disruption of aTS genes does not affect parasite infectivity and escape from the parasitophorous vacuole but resulted in impaired differentiation of amastigotes (AMA) into tissue culture derived trypomastigotes (TCT). Moreover, parasites lacking aTS are unable to establish infection even in the highly susceptible IFN-g knockout mice. To further investigate the role of aTS, we performed *in vitro* infection assays with wild type (WT) and aTS knockout (aTSKO) parasites in two cell models: HeLa (non-phagocytic) and THP1 (phagocytic). Our results confirmed that the lack of aTS does not limit parasite internalization and AMA multiplication in both cell types, but drastically affect release of TCT. Since it was previously shown that several genes associated with the immune response, particularly, type I IFN response are up-regulated in mammalian cells upon *T. cruzi* infection, we performed RT-PCR to determine the expression levels of pro-inflammatory cytokines in cells infected with WT and aTSKO parasites. Compared to cells infected with WT parasites, infection with aTSKO resulted in significantly lower levels of IL-1-β, IL-1-α, IL-6 and CXCL8. To further understand the role of aTS in the egress of the parasite, we developed a strategy to heterologous express aTS in the cell before the infection with aTSKO parasites. **Supported by:**CNPq, CAPES, INCTV **Keywords:**Active trans-sialidases (aTS);host cell egress;pro-inflammatory cytokines.

HP-06 - **IMMUNE RESPONSE MODULATION IN CHRONIC CHAGAS DISEASE AND MHV-3 BETACORONAVIRUS COINFECTION: ROLE OF SOCS-2**

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The balance of immune response (IR) is essential for the survival of both *Trypanosoma cruzi* and host during the acute phase of Chagas Disease (CD). Sars-cov2 infection can causes unbalanced IR associated to pulmonary and systemic damage in its host. Suppressor Cytokine Signaling (SOCS) 2 is an important regulator of the innate and adaptive response to various infections, such as viral and parasitic, but its role during chronic CD and MHV-3 infection are unknown. Our aim was to evaluate the role of SOCS2 during the chronic CD, MHV3, and coinfection chronic CD/MHV3 in mice. C57BL/6 (WT) and SOCS-2 (KO) animals were infected with trypomastigotes forms (Y strain), and after reaching the chronic CD (100 days), was co-infected or not with MHV-3. Deficiency of SOCS2 increased the gut and pulmonary tissue damage at chronic CD and acute MHV3 infection, respectively. During chronic CD, the coinfection with MHV3 increased the gut and cardiac tissue damage in SOCS2 KO mice when compared with WT counterparts. Moreover, a greater parasitism in the colon and higher viral load in the lung during co-infection was observed in SOCS-2 KO mice. The profile of infiltrating cells analyzed by flow cytometry demonstrated that the absence of SOCS2 in chronic CD increased in lung and gut the TNF production by macrophages and IFN-g by CD8 T cells and reduces IL-10 by Tregs. Absence of SOCS2 in MHV-3 infection increased TNF and reduced IL-10 production by all innate cells analyzed, and increased CD4- and CD8 T cells-producing IFN-g in both organs; in lung was observed an increased production of IL-17 by CD8 T cells. Deficiency of SOCS2 during coinfection was marked by a reduction in IL-10 production by all innate cells and lymphocytes analyzed, and by increased CD4- and CD8 T cells-producing IFN-g in lung and gut. In summary, SOCS-2 is essential to modulate the immune response and progression of pathogenesis, especially in co-infection by beta coronavirus MHV-3 in the chronic phase of CD. **Supported by:**CAPES, CNPQ e FAPEMIG **Keywords:**SOCS2;CHAGAS DISEASE;MHV-3.

HP-07 - **Study of the molecular mechanisms of sexual differentiation in *Toxoplasma gondii***

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Toxoplasmosis is a disease of worldwide distribution caused by the intracellular parasite *Toxoplasma gondii*, causing high morbidity and mortality in the human population and farm animals. *T. gondii* has a complex life cycle, with asexual and sexual stages. During sexual differentiation, male and female gametes combine to form diploid zygotes that become encysted and provide the parasite with a window of opportunity for genetic admixing, a crucial step in the generation of genetic diversity. These mechanisms are of particular importance in our continent whereby genetic hypervariability is associated with exacerbated virulence phenotypes. Despite its inherent epidemiological importance, the fundamental aspects of sexual differentiation of *T. gondii* are still unknown. This is mainly due to sexual stages occurring only in the intestinal epithelium of members of the *Felidae* family, and access to them is limited. However, new technological breakthroughs have made it possible to mimic the molecular fingerprints that trigger the process, allowing it now to be modeled *in vitro*. In this context, we have pursued the study of *T. gondii* gametogenesis capitalizing on the unprecedented opportunity to generate these stages in 2D cell culture in non-felid cells. We have optimized an enrichment protocol of pre-sexual and sexual stages of *T. gondii* in traditional 2D cell culture, where we have mapped the increase in sexual-stages specific gene expression patterns by qRT-PCR and determined the subcellular localization of specific protein markers of these stages by indirect immunofluorescence. This will serve as a starting point for bettering our understanding of sexual stages, whose molecular and cellular aspects are so far unknown. Ultimately, will serve as a kick-off point for obtaining data essential to the rational design of strategies to prevent horizontal transmission of *T. gondii* and its genetic recombination in infected cats. **Supported by:**Pasteur network/ Ministerio de Educación y Cultura, Uruguay. **Keywords:**Toxoplasma gondii;horizontal transmission;sexual differentiation.

HP-08 - **Determination of *Toxoplasma gondii's* genetic variability in infections during pregnancy in Uruguay**

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The paradigm for transmission and manifestations of human toxoplasmosis arose based on clinical data and experimentation in mice using strains of *Toxoplasma gondii* circulating in Europe and North America known as "typical." However, we now understand that the severity of human toxoplasmosis depends on the genotype of the parasite, which is closely linked to its geographic origin. Worldwide genotyping studies describe a higher incidence of atypical strains in South America, but this concept is mainly based on data obtained in Brazil, Colombia, and Argentina. Pioneering work in Uruguay described the existence of "atypical" serotypes in patients and the isolation of a genetically atypical strain of Uruguayan origin in France. Based on this, we hypothesize that there is ample genetic variability of circulating *T. gondii* strains in humans in our country. To address this, we genotyped *T. gondii* strains acquired during pregnancy, as determined by the patient's seroconversion detectable during routine pregnancy checkups. We amplified parasite DNA from peripheral blood, placental tissue, and umbilical cord blood from patients. *T. gondii* DNA was detectable in 14 of 21 patients. Positive samples were genotypes using in silico PCR-RFLP of nine polymorphic genetic markers. The presence of "atypical" strains was evidenced in 7 samples. We are currently advancing in their genetic characterization and pursuing the isolation of circulating strains with the aim of correlating genotype with phenotype. **Keywords:**Toxoplasma gondii;Pregnancy;Vertical transmission.

HP-09 - **Identification and characterization of iron metabolism-related proteins in *Leishmania amazonensis* glycosomes**

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The protozoan parasites of the genus *Leishmania* are the causative agents of a group of diseases collectively known as leishmaniases that affect millions of people worldwide. *Leishmania* life cycle includes invertebrate and vertebrate hosts. In the vertebrate host, the parasites survive and replicate inside macrophages, despite the defense arsenal of these cells. One of the critical conditions found by *Leishmania* in the macrophage is the lack of various nutrients, such as iron, an essential cofactor of several enzymes. Besides, it was also shown that iron deprivation modulates the expression of a series of genes whose function is still unknown. Among these, we found conserved genes containing predicted glycosomal addressing signals (PTS1 e PTS2). Therefore, our goal is to identify and characterize genes involved in the transport of iron into *Leishmania* glycosomes, which are trypanosomatids unique organelles. *In silico* analysis of 576 *Leishmania* transcripts modulated by iron deprivation revealed 11 putative genes containing predicted glycosomal addressing signals and transmembrane domains. By immunofluorescence, we confirmed the glycosomal compartmentalization of the proteins encoded by 2 of these genes in promastigotes: a putative gene encoding a hypothetical multipass protein, and an *aquaglyceroporin* (*aqp1*). Then, we used the CRISPR/Cas9 strategy to generate full and partial knockouts, and complemented strains, as confirmed by PCR analyses. The overexpression and knockout of these genes impacted promastigotes *in vitro* growth. Moreover, phenotypic analyses revealed that *aqp1* is implicated on parasite partial resistance to trivalent antimony (SbIII). In-depth characterization of these mutant strains will contribute to elucidate the mechanisms involved in the metabolism and transport of iron into *Leishmania* glycosomes and may indicate new targets for the development of chemotherapeutic agents for the treatment of leishmaniases. **Supported by:**FAPESP 2022/04551-0 **Keywords:**Gene editing;Drug resistance;Immunofluorescence.

HP-10 - **Exploring the function of RNA modifications during *Leishmania* stage differentiation**.

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The *Leishmania* life cycle involves promastigotes present in the insect vector and amastigotes present in the mammalian host. Adapting to these hosts requires not only morphological but also translation and gene expression changes. Gene expression regulation in Leishmania happens mainly at the post-transcriptionally level by mechanisms involving mRNA stability/degradation. Several post-transcriptional modifications of RNA molecules have been described and are widely recognized as an additional mechanism for regulating gene expression. Among these modifications, N6-methyladenosine (m6A) and N1-methyladenosine (m1A) are the most abundant and present in RNAs from different organisms. m6A is add by the methyltransferases METTL3/METTL14 and removed by FTO/ALKBH5 demethylases. Although METTL3 orthologue is absent in trypanosomatids, it was demonstrated the presence of m6A in *Trypanosoma brucei* and its impact in regulating VSG genes. To study the role of m6A in the different *Leishmania* parasite stages we selected five species belonging to different subgenera (*Leishmania mexicana, L. amazonensis, L. infantum, L. major and L. braziliensis*), in order to demonstrate the presence of m6A. Using total RNA samples of the procyclic forms of each species, dot-blot assays with the specific antibody against m6A were performed and we found that in *L. mexicana* the levels of m6A were higher compared to other species, followed by *L. amazonensis and L. infantum*. Based on this data we choose L. mexicana to quantify m6A in the parasite stages, experiments that are in progress. Thus, with these analyses, we will help to understand the processes involved in gene expression regulation in *Leishmania*. **Supported by:**FAPESP; 2023/02341-1 **Keywords:**Leishmania;m6A;epitranscriptome.

HP-11 - **Comparative systems immunology of non-human primate infection with *Plasmodium coatenyi* and *Plasmodium cynomolgy***

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Non-human primates can be infected with P. coatenyei (Pco) and P. cynomolgi (Pcy), which are similar to human infections with P. falciparum and P. vivax infection, respectively. In this context, the Malaria-Host Pathogen Interaction Center (MaHPIC) consortium set up experimental infections and acquired longitudinal data from Macaca mulatta infected with those Plasmodium species for 100 days. The datasets include parasitological and clinical laboratory data, immunological, transcriptomics, metabolomics, proteomics, and lipidomics data. We sought to compare the non-human primate response to infection by different Plasmodium species by analyzing and integrating different types of data. First, we evaluated the dynamics of parasitemia and laboratory clinical data for each individual experiment along the 100 days of infection. Pco induced high parasitemia during acute infection and developed chronic parasitemia that persisted along the 100 days. In contrast, Pcy induced high parasitemia during acute infection, but parasites were cleared from blood after 25 days. This species is known to form hypnozoites, which can cause relapses latter after acute infection is cleared. There were relapses after 50 days and latter after 80 days of infection. Whole blood cell counts increased and remained high along the course of infection with Pco, whereas Pcy induced dynamic changes along the infection, but counts seemed to be comparable to baseline at the end of 100 days. Pco infection reduced red blood cell (RBC) counts that remained low along the infection, while Pcy induced acute RBC loss that recovered with time. The same pattern was observed for hemoglobin and hematocrit levels. Infections induce different patterns of granulocyte, monocyte and lymphocyte counts along 100 days of infection. Taken together, our preliminary results suggest that M. mulatta engages different cellular and molecular responses that depend on the Plasmodium species. **Supported by:**Instituto Serrapilheira, CNPq, FUNAPE **Keywords:**Malaria;Systems Biology;Non-Human Primate.

HP-12 - **Comparative study of biological and molecular characteristics of susceptibility to miltefosine by *Leishmania (L.) infantum* infecting dogs in Florianópolis, Santa Catarina, Brazil**

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Visceral leishmaniasis (VL) is an endemic zoonosis in Brazil, where domestic dogs are urban parasite reservoirs. Between 2010 and 2023, over 900 autochthonous canine VL and 5 human cases were reported in Florianópolis (SC). In this study, we have assessed biological and molecular characteristics of *L. infantum* isolated from naturally infected dogs, before (T0) and after (T1) treatment with miltefosine (MIL). The growth kinetics of all isolated strains was similar but distinct from the PP75 reference strain. Strains isolated before and after *in vivo* treatment showed distinct infectivity to THP-1 cells. No differences were observed *in vitro* for the MIL IC_{50} for both amastigotes and promastigotes (T0 and T1). The transcription of genes associated with susceptibility to MIL revealed a positive modulation in promastigote treated *in vitro*. No genetic resistance profile to MIL based on the LiMT and LiRos3 genes was observed. Proteomic analysis of MIL treated and untreated *L. infantum*-infected THP-1 carried out by MS resulted on the identification of a total of 4,729 unique proteins, from which 4,235 were from THP-1 and 494 from *L. infantum*, representing 5.8% of the predicted proteins in the JPCM5 reference strain genome. The function was assigned to 99% of these proteins, most of which being cytoplasmic (44.3%), followed by mitochondrial subcellular localization (19.9%). The signal peptide was detected for 2% of these proteins, and 7.3% have at least one transmembrane domain. A total of 225 proteins were shared among the six strains despite the experimental conditions. Proteins with significant differential abundance did not show alteration associated with the mechanism of action or resistance to MIL. Six B-cell epitopes from five proteins were identified, with five shared among *Leishmania* spp. species, and a single *L. infantum*-specific epitope. Despite some strain-specific variations, neither phenotype nor genotype associated with resistance to miltefosine was observed. **Supported by:**CNPq e CAPES **Keywords:**Canine Visceral Leishmaniasis;Miltefosine;Intracellular Amastigote.

HP-13 - **Preliminary structural analysis of the Venus Flytrap domains of the ESAG4 receptor-like adenylate cyclase ectodomain in *Trypanosoma brucei***

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Trypanosoma brucei, a protozoan parasite responsible for causing Sleeping Sickness, a neglected tropical disease prevalent in Africa, possesses a large family of transmembrane receptor-like adenylate cyclases (RACs)[1], which are the only known signalling receptors at the cell surface. All RACs possess a conserved architecture, consisting of a large receptor ectodomain composed of two Venus Flytrap domains (VFT1 and VFT2), a transmembrane helix, and a cytosolic catalytic domain with cyclase activity. We have previously shown that activation of trypanosomal RACs expressed in the mammalian host, triggered by mild acid stress, leads to the production of large amounts of cAMP. This elevated cAMP level inhibits TNF-α synthesis in host myeloid cells, allowing immune evasion during the early stage of infection[2]. Nevertheless, the molecular mechanisms triggering the activation of RACs through the ectodomain and the identification of their putative ligands remain unknown. Although the surface architecture of the bloodstream parasite, imposed by the VSG coat, may play a critical role in regulating cyclase activity[3], the specific ligands that bind to VFT domains are currently unknown. This project aims to address this gap by identifying the ligands for a bloodstream-specific RAC ectodomain (named ESAG4) and further characterizing the structures of the ectodomain-ligand complexes. To characterize the ligands, several VFT2 soluble protein constructs were produced in bacteria and purified using affinity and size exclusion chromatography, followed by quality control assessments using biophysical techniques. The structural characterization of ESAG4 extracellular sensor domain and its interaction with ligands will potentially provide valuable insights for the development of novel strategies against trypanosome infections. 1. Alexandre S., et al. *Mol Biochem Parasitol.* 43, 279-88 (1990) 2. Salmon D., et al. *Science*. 337, 463-6 (2012) 3. Rolin S., et al. *J Biol Chem*. 271,10844-52 (1996) **Supported by:**CNPQ processo: 870092/1997-9 **Keywords:**Trypanosoma brucei;Adenylate cyclase;Structural biology.

HP-14 - **Modifying to adapt: the impact of lysine deacetylases in *Leishmania* parasite stage differentiation**

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Protein acetylation has been implicated in the regulation of essential cellular processes in diverse organisms. Proteomic analysis from our group revealed differential protein acetylation among the three main *Leishmania mexicana* stages. Thus, to expand our knowledge on how changes in protein acetylation affect *Leishmania* differentiation, we generated knockout and fluorescent endogenous tagged parasite strains using the CRISPR-Cas9 system of all lysine deacetylases of the parasite (DACs 1, 3, 4 and 5). We obtained only single-knockouts for DAC1/3, and null mutants for DAC4/5, suggesting that DAC1-3 are essential for procyclic stages. Using the fluorescent parasites, we found that DAC1/5 are cytoplasmatic, while 3/4 have nuclear localization in all stages. Phenotype analyses using the mutant parasites showed that: **i)** DAC1/3/5 affect procyclic multiplication; **ii)** DAC3/5 affect procyclic to amastigote stage differentiation; **iii)** all DACs are important during amastigotes to procyclics differentiation; **iv)** DAC1/5 directly affect *in vitro* differentiation of procyclic to metacyclic infective forms **v)** DAC3/5 impacts metacyclogenesis in experimental *in vivo* infection of *Lutzomyia longipalpis*; **vi)** *in vitro* infection with BMDMs showed decreased in the survival and proliferation for all DAC mutants; **vii)** A similar scenario was observed in the Balb/c *in vivo* infection assays for DAC5 mutants, with no apparent lesion development compared to parental parasites. Taken together, these results indicate that regulation of protein acetylation levels might be important for *L. mexicana* differentiation and pathogenesis, opening the opportunity to explore DACs as potential drug targets. **Supported by:**FAPESP 2021/13477-6 **Keywords:**Leishmania mexicana;acetylation;CRISPR.

HP-15 - **Disruption of the inositol phosphorylceramide synthase gene affects *Trypanosoma cruzi* differentiation and infection capacity**

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Sphingolipids (SLs) are essential components of all eukaryotic cellular membranes. In fungi, plants and many protozoa, the primary SL is inositol-phosphorylceramide (IPC), which is absent in mammals. *Trypanosoma cruzi* is a protozoan parasite that causes Chagas disease (CD), a chronic illness for which no vaccines or effective treatments are available. IPC synthase (IPCS) has been considered an ideal target enzyme for drug development because IPCS null mutants are not viable and the enzyme activity has been described in all parasite forms of *T. cruzi*. Furthermore, IPCS is an integral membrane protein conserved amongst other kinetoplastids, including *Leishmania major*, for which specific inhibitors have been identified. Using a CRISPR-Cas9 protocol, we generated *T. cruzi* knockout (KO) mutants in which both alleles of the IPCS gene were disrupted. We demonstrated that the lack of IPCS activity does not affect epimastigote proliferation or its susceptibility to compounds that have been identified as inhibitors of the *L. major* IPCS. However, disruption of the *T. cruzi* IPCS gene negatively affected epimastigote differentiation into metacyclic trypomastigotes as well as proliferation of intracellular amastigotes and differentiation of amastigotes into tissue culture-derived trypomastigotes. In accordance with previous studies suggesting that IPC is a membrane component essential for parasite survival in the mammalian host, we showed that *T. cruzi* IPCS null mutants are unable to establish an infection *in vivo*, even in immune deficient mice. **Keywords:**Sphingolipids;IPC synthase;Inositol-phosphorylceramide.

HP-16 - **Molecular Detection and Genotyping of *Blastocystis spp* in samples from patients suffering from inflammatory bowel disease in Uruguay.**

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Blastocystis spp is frequently detected in routine diagnosis of inflammatory bowel diseases (IBDs). However, the association between the presence of the agent and its impact on the pathology of these diseases is ill-understood. Moreover, whether there is a correlation between specific genotypes of *Blastocystis spp* and the histopathological manifestations of IBDs, has not been clearly established. Here, we set out to determine the *Blastocystis spp* genotypes present in patients suffering from inflammatory bowel diseases in Uruguay. First, we optimized tools for DNA extraction from fecal samples, and for molecular detection of the agent by PCR amplification of its SSU rRNA. Secondly, to gain insight into the genotypes present in the country, we assessed which *Blastocystis* subtypes were found in these samples by Sanger sequencing and BLAST analyses. We have additionally optimized the culture and cryopreservation of these parasites, generating a biobank of autochthonous isolates. Preliminary results on our study populations suggest that *Blastocystis* is 40% more likely to be detected in patients suffering from IBD than in control patients or patients suffering from unrelated intestinal diseases. The genotype found most often corresponds to ST6 which was detectable in 3 out of 4 positive samples. Overall, our results suggest that patients suffering from IBD could be especially vulnerable to colonization by specific *Blastocystis* genetic subtypes. To gain mechanistic insight into the clinical significance of these findings, we are currently analyzing the correlation between these genetic subtypes, the clinical outcomes in IBD patients, their inflammatory profiles and their associated changes in intestinal microbiota. **Keywords:**Blastocytis spp;Inflammatory bowel diseases;Parasite.

HP-17 - **Strategies for the identification of genes involved in the Vertical Transmission of *Toxoplasma gondii***

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The most devastating consequences of toxoplasmosis are connected to the ability of *Toxoplasma gondii* to access and infect vital anatomical sites such as the placenta. Infection during pregnancy can cause miscarriages, stillbirths, premature births and babies born with severe debilitating neuropathies. In in Uruguay *T. gondii* is the causative of 75% of abortions from infectious etiology in sheep, causing millionaire loses every year. Despite its importance, the mechanisms underlying transplacental transmission are poorly understood. Genome wide CRISPR screens are powerful tools to identify genes required for processes under selective biological pressure. The strategy involves a parasite population that expresses Cas9 and a single gRNAs per cell, targeting selected genes that are further subjected to a “challenge”. The readout of the experiment involves comparative whole genome sequencing pre and post challenge. Through the identification of gRNA profiles of each population genes that are lost or not upon the specific challenge are postulated as essential or dispensable candidates. In this project, we propose to use this approach to identify apicomplexan factors required for surviving the biological pressures upon vertical transmission strategies. The identification of parasite genes that are essential for transplacental passage may contribute to the development of prevention, control, and prophylaxis strategies to mitigate congenital toxoplasmosis and other related apicomplexan diseases. Furthermore, the work pipeline and resourced developed within this project represent a potential starting point for additional *in vivo* or *in vitro* experiments addressing other biological questions regarding the pathogenic mechanisms of parasites in this phylum. **Keywords:**Vertical transmission;Placental tropism;CRISPR-Cas9 genome wide libraries.

HP-18 - **Unveiling the Infectiveness-associated Proteins of *Trypanosoma cruzi* G and CL Strains: Insights from Proteomics, Phosphoproteomics, and N-Linked Glycoproteomics**

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Trypanosoma cruzi extracellular amastigotes (EAs) are able to infect cultured cells and animals, establishing a sustainable infective cycle. EAs are either prematurely released from infected cells or generated by the extracellular differentiation of released trypomastigotes. Among the EA stage, *T. cruzi* I strains (such as the G strain) are more infective than *T. cruzi* II and VI strains (such as the Y and CL strains). Here we aimed to perform a comprehensive proteomics – including phosphoproteomics and N-linked glycoproteomics - of both EA G and CL strains. EA samples were obtained by differentiation of TCT forms in acid medium (pH 5.8), with 90-95% purity. Samples were lysed and digested with trypsin, labeled with TMT10plex (ThermoFisher) and enriched for phosphopeptides and glycopeptides (TiO2 and deglycosylation with PNGaseF/SialidaseA). Sample complexity was reduced by pre-fractionation using high pH reverse phase chromatography (HpH-RP; Dionex Ultimate 3000). The LC-MS/MS analyses were done using the Exploris 480 mass spectrometer (ThermoFisher). Results were analyzed in the Maxquant program (V. 1.8.6.0) using a database of 47847 sequences for the G and CL strains, obtained from TritrypDB database. The data was statistically analyzed in Perseus (V. 1.8.6.0) and DanteR (V. 1.0.0.10) programs. A total of 5532 proteins were identified from the total proteome, 785 of these were differentially regulated in the G strain, and 561 in the CL strain (P-value < 0.01). For the phosphoproteome and glycoproteome, 4959 phosphopeptides (2298 proteins) and 935 N-glycopeptides (666 proteins) were identified, respectively. Differentially regulated proteins play roles in the invasion process and establishment of infection in the host, such as trans-sialidase and mucin families, as well as calpain, cruzipain, and mevalonate kinase. This study shows, for the first time, the proteins potentially associated with the strains’ infectiveness. **Keywords:** Extracellular Amastigotes T; cruzi;Phosphoproteomics;Gycoproteomics.

HP-19 - **DEVELOPMENT OF RNA VACCINES FOR LEISHMANIASIS AND COMPARISON WITH VACCINES BASED ON RECOMBINANT ANTIGENS**

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Despite intensive efforts and studies in animal models indicating variable levels of protection achieved by immunization with defined subunit vaccines, to date, no vaccine for human leishmaniasis is available. Because, as demonstrated during COVID-19 pandemic, RNA vaccines have proven to be a technological breakthrough, we decided to develop an RNA vaccine and compare its protection level with the immune response and protection obtained with a recombinant vaccine based on the same antigen. The protein associated with kinetoplast (PAK), identified after two-dimensional gel analysis and mass spectrometry of L. amazonensis proteins using serum from mice immunized with total extract of the parasite, was selected as a target antigen. Immunization of mice with recombinant PAK, named DTL8, was able to generate a Th1 response that partially reduced the parasite load of animals after challenge with L. infantum. In vitro transcribed DTL8 RNA containing the appropriated 5' and 3' UTRs and a poly-A tail encapsulated in a lipidic nanoparticle (LNP) formulation was used to immunize C57BL/6 and BALB/c mice. In contrast to a weak antibody response in BALB/c mice, immunization of C57BL/6 mice with DTL8 RNA resulted in the induction of higher levels of antibodies compared to immunization with recombinant DTL8. To investigate whether the composition of the LNP influences antibody production, we immunized mice with the DTL8 RNA encapsulated in our LNP formulation or in a LNP formulation present in the Moderna Covid-19 RNA vaccine, but no differences in the antibody levels were observed. After challenging immunized BALB/c mice with L. infantum, only animals immunized with recombinant DTL8 were partially protected against the infection, a result that is probably due to the low antibody levels observed in this animal model. To *evaluate the protection against infection* with L. amazonensis, we are currently immunizing C57BL/6 mice with DLT8 RNA and with the recombinant antigen. **Supported by:**Cnpq
Keywords: Leishmaniasis;RNA vaccine;LNP formulation.

HP-20 - **Selective and synergistic leishmanicidal action: an evaluation of repositioned and new compounds isolated and combined**

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The search for safe and orally administered therapies persists as a demand for cutaneous leishmaniasis. Drug repositioning and combination are promising strategies to reduce development time, cost, and risk, as well as increase efficacy and reduce toxicity. Thus, we investigated the *in vitro* activity of POD1, AM and NAM (under patent secrecy) isolated and combined. For this, promastigotes of *Leishmania amazonensis* PH8 and J774 macrophages untreated or treated with different concentrations of compounds were used, serially diluted in 96-well microplates. The Alamar Blue assay was used to assess cell viability after 24h and to determine the IC_{50}, CC_{50} and selectivity index (SI). Combinations were performed using the fixed proportions method to determine FIC and $\bar{x}\Sigma FIC$, analyzed according to Odds, and plotted in isobolograms. The IC_{50} values of POD1, AM and NAM in promastigotes were 317.06, 61.44 and 5.42 µM, respectively. While the CC_{50} in J774 cells were 1187.00, 241.50 and 194.20 µM, in that order. Therefore, the following SI were obtained: SI_{POD1} = 3.74, SI_{AM} = 4.0, SI_{NAM} = 35.93. In combination, the IC_{50} of POD1 (19.95 µM), AM (1.37 µM) and NAM (0.90 µM) reduced by 16, 45 and 6-fold as compared to their isolated actions. All POD1:AM combinations based on FIC_{50} were synergistic (≤ 0.5), as were the 3:2 and 2:3 POD1:NAM ratios. High synergistic activities (≤ 0.1) were achieved in combinations 4:1 in FIC_{25} POD1:AM and 3:2 in FIC_{75} POD1:NAM. The other proportions were also synergistic or additive (> 0.5 or ≤ 4.0), with no antagonistic combination (> 4.0). Analysis of $\bar{x}\Sigma FIC_{50}$ demonstrated that the combination POD1:AM was synergistic (0.29) and POD1:NAM additive (0.90). Together, our data corroborate the potential of combinations to reduce concentrations and increase the leishmanicidal effect. **Supported by:**Fiocruz, IOC, CNPq, CAPES, FAPERJ, PPSUS **Keywords:**Leishmaniasis;Repositioned drugs;Drug combination.

HP-21 - **PI(3,4,5)P3-dependent allosteric regulation of repressor-activator protein 1 (RAP1) controls antigenic switching in African trypanosomes**

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African trypanosomes evade host immune clearance by antigenic variation expressing a surface coat of variant surface glycoproteins (VSGs). They transcribe one out of hundreds of VSG genes at a time from telomeric expression sites (ESs) and periodically change the VSG expressed by transcriptional switching or recombination. The mechanisms underlying the control of VSG switching and its developmental silencing remain elusive. We report that telomeric ES activation and silencing entail an on/off genetic switch controlled by a nuclear phosphoinositide signaling system. This system includes a nuclear phosphatidylinositol 5-phosphatase (PIP5Pase), its substrate PI(3,4,5)P3, and the repressor-activator protein 1 (RAP1). Cross-linking and mass spectrometry analysis revealed that these proteins are part of an interaction network including DNA/RNA-binding proteins, protein kinases, phosphatases, and nuclear lamina proteins, implying an ES signaling complex compartmentalization at the nuclear periphery. ChIP-seq assays show that RAP1 binds silent ESs at sequences flanking VSG genes, namely 70 bp and telomeric repeats. Gel shift and binding kinetics confirmed that RAP1 binds directly to 70 bp or telomeric repeats, which occurs via its Myb and Myb-like domains. In contrast, we found a villin headpiece domain in the N-terminus of RAP1 and show this region binds PI(3,4,5)P3. PI(3,4,5)P3 acts as an allosteric regulator of RAP1, and its binding removes RAP1 from 70 bp or telomeric repeats. Transient expression of catalytic inactive (D360A/N362A) PIP5Pase, which cannot dephosphorylate PI(3,4,5)P3, results in the accumulation of RAP1-bound PI(3,4,5)P3 in bloodstream forms. Importantly, ChIP-seq ad RNA-seq in these cells confirmed the displacement of RAP1 from silent ESs *in vivo*, *resulting* in silent ES transcription and activation of VSG gene switching. The data provides a mechanism controlling reversible telomere silencing essential for the periodic switching in VSG expression. **Supported by:**Canadian Institutes of Health Research grant CIHR PJT-175222 (IC); The Natural Sciences and Engineering Research Council of Canada grant RGPIN-2019-05271 (IC); Fonds de Recherche du Québec - Nature et Technologie grant 2021-NC-288072 (IC); Canada Foundation for Innovation grant JELF 258389 (IC)
Keywords: Antigenic variation;transcription;nucleus.

HP-22 - **In vitro analysis of Anilinoquinazolines: new perspectives for the treatment of toxoplasmosis.**

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Toxoplasmosis is an important neglected disease related to congenital malformations, retinochoroiditis, and encephalitis in immunocompromised patients. Conventional treatment with sulfadiazine and pyrimethamine has limitations, such as toxic side effects, potential cases of resistance, and inactivity against the chronic phase of the infection. Therefore, new, and more selective compounds for the treatment of acute and chronic toxoplasmosis are needed. In this study, we tested the effect of six Anilinoquinazolines derivatives, a new group of compounds derived from quinazoline, which have recently been explored as antineoplasic and antiparasitic drugs.
Different concentrations (1, 2, and 5 µM) of anilinoquinazolines (1A-6A) were tested in an antiproliferative assay with the highly virulent RH strain (Type I) in cultures of NHDF cells infected with tachyzoites for 24h. The cytotoxic effect of these compounds against host cells was evaluated by the MTS/PMS assay using concentrations of up to 10µM for 7 days. The 50% of effective parasite inhibition concentration (IC50) and the 50% cytotoxic concentration for the host cells (CC50) were calculated to obtain the Selective Index (SI), calculated as the CC50/IC50 ratio.
All six compounds tested were active against *T. gondii* tachyzoites with IC50 at the nano and micromolar range (0.73-2.9 µM) after 24h of treatment. For four compounds (1A-4A), SI ranged from 9 to 68. Three of the six compounds (1A, 2A, and 4A) had an IC50 less than 1uM and SI greater than 20, values recommended by the World Health Organization. In addition, the analysis of the physicochemical properties of these compounds showed that they do not violate Lipinsky and Veber's rules, being favorable for future oral use. Thus our results confirmed that anilinoquinazolines control the growth of tachyzoites of *T. gondii,* pointing to a new class of compounds with the potential for developing a future treatment for toxoplasmosis. **Keywords:**Toxoplasma gondii;toxoplasmosis;treatment.

HP-23 - **Screening of the antileishmanial activity of 103 extracts of endophytic fungi collected in the Amazon rainforest**

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The treatment for leishmaniasis has few therapeutic options and recommended drugs are highly toxic, costly, and the treatment has long-lasting. Thus, the discovery of new therapeutic options has global importance. This work aimed to evaluate the *in vitro* anti-*Leishmania* potential of 103 extracts of endophytic fungi isolated from *Arrabidaea chica* (Bignoniaceae), popularly known as Crajiru. The fungi were isolated from the leaves and branches of different trees in the Amazon rainforest and are deposited in the Microbiological Collections Center (CCM/UEA). The screenings were carried out with *Leishmania (L.) amazonensis* (MHOM/BR/1973/M2269). The promastigotes forms were incubated for 24h with each extract, and the cell viability was assessed with PrestoBlue® dye. Extracts that showed the lowest IC_{50} values in the promastigote test were evaluated for their ability to eliminate intracellular amastigote form in infected murine macrophages. Parasite cell cycle alteration and cell death were performed by flow cytometry using Annexin V markers and Propidium Iodide. The IC_{50} values of the 103 tested extracts ranged from 67.1±1.66 µg/mL±SD to values greater than 1250 µg/mL, while the reference drug Pentamidine showed an IC_{50} value of 0.38±0.09 µg/mL±SD. Two extracts showed lower IC_{50} values (67.1±1.66 and 71.4±3.06 µg/mL±SD) and maintained their stability in the three independent experiments. Cytotoxicity tests were performed with murine macrophage RAW 264.7, showing CC_{50} values thirteen times higher than the IC_{50} values for the extracts and up to six times for the standard drug, showing greater toxicity to the parasite and not the host cell. These extracts showed a slight antioxidant activity and no antimicrobial effects. Identification of chemical compounds present in the extracts, their mechanism of action, and *in vivo* analyses will contribute to better evaluating the possibility of using these extracts/derived molecules in therapy for cutaneous leishmaniasis. **Supported by:**FAPESP/CNPQ/FAPEAM
Keywords: Leishmania;extracts;endophytic fungi.

HP-24 - ***In vitro*** **susceptibility of macrophages obtained from *T. crassiceps* parasitized mice to *Leishmania major* and *L. braziliensis***

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For the development of leishmaniasis, macrophage must be infected by parasites. While the killing of Leihmania is associated with classically activated macrophages (CAMs), the survival and dissemination of parasites are associated with the presence of alternatively activated macrophages (AAMΦ). AAMΦ can be obtained by different methods, including recuperation of cells from helminthic infected mice. In this work, macrophages were obtained from mice injected with Thioglycolate (TG) or mice previously infected with Taenia crassiceps cysticerci (TC). The cells were characterized and used to evaluate the susceptibility to Leishmania (L.) major and Leishmania (V.) braziliensis infection. TC macrophages produced less NO after LPS stimulus and presented higher arginase activity when compared to TG macrophages. Additionally, TC macrophages were more susceptible to infection by L. (L.) major and L. (V.) braziliensis when compared to TG macrophages. The higher susceptibility of TC macrophages to Leishmania infection did not allow L. (L.) braziliensis to proliferate inside macrophages when infected macrophages were cultured for 10 days. In contrary, L (L.) major proliferated inside TG and TC infected macrophage in the same period. Our data demonstrate that AAMΦ derived from helminthic infection are more susceptible to Leishmania infection, but control proliferation of L. (L.) braziliensis. **Supported by:**FAPEG (202110267000628); CAPES (001); INCTMCTI/CNPQ/Universal (14/2014)
Keywords: Leishmania;Cysticerci;Alternatively activated macrophages.

HP-25 - **CRK3 role in modulation pathways of cell cycle and metacyclogenesis in *Trypanosoma cruzi***

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Trypanosoma cruzi, a clinically relevant protozoan parasite responsible for Chagas disease, undergoes several phenotypic and biochemical changes throughout its life cycle, indicating an intricate regulation of its cellular mechanisms. Understanding the interruption of the cell cycle in the infective (quiescent) forms and its reactivation in replicative stages is of great interest not only in the field of cell cycle research but also in areas such as pharmacology, as dormant amastigote parasites exhibit resistance to current treatments. Therefore, this study aims to characterize how CRK3 (Cdc2-related kinase 3), a kinase proven to take part in the cell cycle, modulates the cell cycle and life cycle of *T. cruzi* using techniques such as CRISPR/Cas9 DNA editing for knock-in and knock-out, cell cycle synchronization with hydroxyurea, immunofluorescence, and in vitro metacyclogenesis. Two *T. cruzi* strains were generated, with one strain expressing the Myc tag at the N-terminal of CRK3, while the other strain has a Myc tag at the C-terminal of CRK3. We validated these strains through sequencing, western blot, and growth curve analysis. With these strains, we observed no difference in the expression of CRK3 throughout the cell cycle of epimastigotes. However, in metacyclic trypomastigotes, the expression of CRK3 significantly decreased or was completely absent during metacyclogenesis. CRK3 was predominantly localized in the cytoplasm but exhibited discrete nuclear localization in some parasites. There was no difference in expression and localization between the two strains. These findings suggest that the regulation of CRK3 occurs predominantly through post-translational mechanisms, which involve nuclear-cytoplasmic shuttling and the involvement of other regulatory proteins, such as cyclins, CRK inhibitors, and possibly other unidentified proteins.
Supported by:FAPESP - 2022/05264-5 **Keywords:**CRISPR/Cas9;Trypanosoma cruzi;Ciclo Celular.

HP-26 - **COMPARISON BETWEEN LIGHT MICROSCOPY COUNTING AND PROMASTIGOTE RECOVERY ASSAY TO ASSESS PARASITE BURDEN IN IN VITRO INFECTIONS WITH LEISHMANIA.**

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INTRODUCTION: Light microscopy counting (LMC) and promastigote recovery assay (PRA) are classical methods of assessing parasite burden (PB) in *in vitro* infections with *Leishmania* parasites **OBJECTIVE:** Analyse the limitations and strengths of each PB assessment method using *in vitro* infection of macrophages with *L. major* (Lm) or *L. braziliensis (Lb)*. **METHODS:** Macrophages from thioglycolate-elicited BALB/c mice were infected with *Lb* or *Lm* to evaluate PB 3h, 72h, 144h and 216h post infection (PI). LMC assay was conducted after staining cells to access percentage of infected cells (%IC) parasites per infected macrophage (P/C) infection index (II) and infection score. PRA was evaluated after incubation of infected macrophages in grace's insect medium at 25^0C for 3 days (*Lm*) or 5 days (*Lb*) for each time PI. **RESULTS:** The LMC method was efficient in distinguishing the susceptibility of BALB/c macrophages to *Lm* infection evidencing PB growth from 3h to 72h, 144 and 216h PI respectively (%IC= 54,38±10,41%; 65,67±11,24%; 65,52±14,43%; 69,32±6,98%) (P/C= 2,05 ±0,58, 3,79±0,83; 4,62±0,91; 7,31±0,85) (II= 108,42 ±25,57; 251,50±78,39; 302,28±79,33; 510,09±99,13). It was also efficient in demonstrating macrophage resistance towards infection with *Lb* species with the decrease of PB from 3h to 144h and 216 PI respectively (P/C = 3,59±0,33; 2,33±0,18; 2,26±0,18) (II= 223,01±58; 107,80±21,71; 101,37±20,06). This method also allowed the evaluation of different cell populations that hosted varying quantities of amastigotes through different score for both parasites. The PRA efficiently demonstrated the contrasting phenomena of PB in infections with both parasite species but with parasite growth only being observed after 216h PI on *Lm* infections and parasite decrease after 144h on *Lb* infections. **CONCLUSION:** Both tests can distinguish the phenomena of susceptibility or resistance of macrophages to different leishmania species, but they offer more valuable information when used together.
Supported by:INCT/MCTI/CNPQ/Universal 14/2014 **Keywords:**Leishmania braziliensis;Leishmania major;Peritoneal macrophages.

HP-27 - **Vps32 plays an important role in endocytic traffic and cell cycle progression in *Trypanosoma brucei***

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Trypanosoma brucei is the causative agent of African trypanosomiasis in humans and cattle. This parasite presents two different developmental stages: the tse-tse fly procyclic form (PCF) and the mammalian bloodstream form (BSF). Stage differentiation is critical for successful life cycle progression, while endocytosis, exocytosis and autophagy are crucial to guaranteeing their survival. Endosomal Sorting Complex Required for Transport (ESCRT) consist of a series of complexes integrated by many proteins responsible for vesicles formation during intracellular transport. In trypanosomatids the most engaging is ESCRT-III, which is highly preserved in eukaryotes. A member of this complex, Vacuolar Protein Sorting 32 (Vps32) plays important roles in cytokinesis and endocytic traffic, as demonstrated in human and yeast models. Previously in our laboratory, we identified the Vps32 orthologue in *T. brucei* (TbVps32) and observed that TbVps32 downregulation decreases endocytosis and affects intracellular transport. In our current work our aim is to further investigate the role of this protein in the PCF stage. To achieve this we have obtained two different cell lines: one that over-expresses TbVps32 under a Tet-inducible regulatory system (HA-TbVps32) and another in which the protein expression can be silenced by an inducible interference RNA system (TbVps32-iRNA). With these cell lines, we have studied TbVps32 localization and dynamics. Furthermore, we are focusing our studies on determining the precise step in which TbVps32 is participating during endocytic traffic. In conclusion, we have demonstrated that both TbVps32 over-expression and silencing impairs cell proliferation, and its downregulation results in severe abnormal nucleus-kinetoplast configurations. Overall we propose that TbVps32 participates in endocytic traffic to the lysosome and is essential for *T. brucei* survival. **Supported by:**Proyectos de Investigación Plurianuales (PIP) 2021-2023. CONICET. Nro. 03073. “Señalización y regulación del ciclo celular en epimastigotes de Trypanosoma cruzi” **Keywords:**ESCRT;TbVps32;procyclic form.

HP-28 - **Advancing Drug Discovery for Leishmaniasis: Targeting the Proteasome 20S with AI-driven Compound Selection**

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Proteasomes are protein complexes that act as the main proteolytic system in eukaryotic cells, degrading unneeded or damaged proteins into small peptides that are recycled as amino acids. If inhibited, proteasomes lead to the accumulation of ubiquitinated proteins and vesicles, triggering an autophagic cell death pathway. Through a collaborative effort together with Atomwise, we have pre-selected potential anti-leishmanial compounds utilizing their proprietary technology, AtomNet®. For target-based hit discovery, a molecular library of million compounds was virtually screened against Leishmania proteasome 20S. A total of 84 potential componds were selected as active against L. infantum. Further phenotypic screening against L. amazonensis and L. braziliensis intracellular amastigotes filtered the 20 most active compounds. Most of the selected compounds were nontoxic to THP-1 derived human macrophages. To confirm proteasome inhibition, a cell-based proteasome 20S activity assay was conducted using two compounds 1 [C1] and 4 [C4]. The assay measured the chymotrypsin-like (CT-L), trypsin-like (T-L), and caspase-like (C-L) proteases activity associated with the proteasome. Marizomib was used as proteasome inhibition positive control. Marizomib presented proteasome inhibition [IC50 = 0.72 to 1.43 nM] associated with CT-L protease activity for all Leishmania species. T-L and C-L protease activities were inhibited in a less expressive manner [IC50 = 2 to 458 nM]. C1 inhibited CT-L and C-L activities with IC50 of 1.47 and 751 nM, respectively; while T-L activity was inhibited by C4 at 1 µM, only on L. amazonensis. This suggests the presence of different targets in L. braziliensis and L. infantum. Since structural analysis revealed similarities among proteasome 20S from different trypanosomatids, these lead compounds could also be tested against human and animal trypanosomiasis. Through collaborative efforts, it is possible to accelerate the drug discovery process. **Supported by:**Fapemig, CAPES, CNPq, Fiocruz
Keywords: Leishmania; Proteasome 20S inhibition;Drug discov.

HP-29 - **Transcriptomic analysis of the Interferon pathways in macrophages during Neospora caninum and Toxoplasma gondii infections**

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Neospora caninum and *Toxoplasma gondii* are obligatory intracellular parasites that affect a wide range of animals. Interferon-gamma (IFN-γ) is known to be fundamental in the immune response against these parasites. However, there is little or no data about the roles of type I and III Interferon pathways in controlling these infections. Therefore, our study aimed to compare, through transcriptome analysis, the differential gene expression of Interferon type I, II, and III pathways during the infection by *T. gondii* and *N. caninum*. For that purpose, we conducted RNASeq analysis of marrow-derived macrophages obtained from C57BL/6 mice, infected with both parasites, for 6 hours, and compared the gene expression of the targeted pathways using TPM (transcriptions per million) values. Using that approach, we found that the expression of most of the genes in the interferon pathways was increased during the infection with *N. caninum*, compared to the non-perturbed or downregulated genes. We observed a distinct profile in response to *T. gondii* infection, with a higher percentage of genes that did not undergo changes in expression. This phenotype was especially noted in type I and III Interferon pathways. Heatmap analysis of single genes divided by pathways further corroborated these observations, showing significant alterations in critical genes. These results highlight that, despite the phylogenetic similarities between these parasites, there are striking differences in host-parasite interactions, which may account for their distinct pathogenesis in mice and other hosts. **Supported by:**CNPq (313761/2020-5), FAPEMIG (RED-0313-16) **Keywords:**Innate immunity;RNASeq;Signaling pathways.

HP-30 - **EFFICACY OF INTRALESIONAL MEGLUMINEANTIMONIATE IN THE TREATMENT OF CANINE TEGUMENTARY LEISHMANIASIS: A RANDOMIZEDCONTROLLED TRIAL**

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Background: Dogs living in areas of *Leishmania (Viannia) braziliensis* transmission may present canine tegumentary leishmaniasis (CTL) characterized by cutaneous or muzzle ulcers as well as asymptomatic *L. braziliensis* infection. It is not clear if dogs participate in the transmission chain of *L. braziliensis* to humans. However, dogs may remain with chronic ulcers for a long time, and as there are no public policies about CTL, these animals die or are sacrificed. **Objective:** We compare the efficacy of intralesional meglumine antimoniate with intralesional 0.9% NaCl solution in CTL treatment. **Methods:** This randomized control study included 32 dogs with cutaneous or muzzle lesions who had *L. braziliensis* DNA detected by PCR in tissue biopsied. Group one received 5ml of intralesional meglumine antimoniate, and group two received 5ml 0.9%NaCl solution, both applied in the four cardinal points on days 0, 15, and 30. Cure was defined as complete healing of the ulcers in the absence of raised borders on day 90. **Results**: There was no difference in animals' demographic and clinical features in the two groups ($p > .05$). While at the endpoint, the cure rate was 87.5% in the group test, in those who received 0.9 NaCl the cure rate was only 12.5% ($p < 0.01$). As important as the high cure rate, the healing time was faster in dogs treated with meglumine antimoniate than in those treated with saline ($p < .001$). **Conclusion:** Intralesional meglumine antimoniate is effective in the treatment of dogs with *L. braziliensis* infection and accelerates the healing time of CTL. **Supported by:**Instituto Nacional de Ciência e Tecnologia em Doenças Tropicais (INCT-DT), CNPq #465229/2014-0 **Keywords:**Leishmania braziliensis;Canine tegumentary leishmaniasis;Treatment of canine tegumentary leishmaniasis.

HP-31 - **THE ROLE OF DOGS IN THE TRANSMISSION OF *Leishmania braziliensis* IN AN ENDEMIC AREA OF AMERICAN TEGUMENTARY LEISHMANIASIS**

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Background: Cutaneous leishmaniasis (CL), caused by *Leishmania braziliensis,* is the most important presentation of American Tegumentary Leishmaniasis (ATL). Little is known about the importance of dogs in the transmission of *L. braziliensis* to humans. **Objectives:** In the present study, we determine the frequency of *L. braziliensis* infection in dogs with cutaneous and mucosal ulcers in Corte de Pedra, Bahia, Brazil, an endemic area of CL, describe the clinical manifestations and histopathologic features, and determine if the parasites isolated from dogs are genetically similar to those found in humans. Also, we determine the prevalence and incidence of both cutaneous tegumentary leishmaniasis (CTL) and subclinical (SC) *L. braziliensis* infection in dogs and evaluated if the presence of dogs with the CL or SC infection is associated with the occurrence of ATL in humans. **Methods:** SC infection and CTL were determined by PCR on biopsied healthy skin or on the ulcer or by detecting antibodies against soluble leishmania antigen (SLA). To determine if *L. braziliensis* infection in dogs was associated with human ATL we compared the occurrence of ATL in homes with or without dogs with CTL or SC infection. **Results:** The PCR was positive in 41 (67%) of animals, and the lesions in the snout, followed by the testicles and ears were the sites where parasite DNA was most detected. There were genotype **s**imilarities between *L.braziliensis* isolates from dogs and humans. The prevalence of SC infection was 35% and of CTL 31%. The incidence of SC infection in dogs was 4.6% and the incidence of CTL was 9.3%. The frequency of ATL in humans in homes with infected dogs was 50% (38/76), while it was 13% (7/56) in homes without evidence of *L. braziliensis* infection in dogs (P<0.01)**. Conclusions**: CTL and SC infection are highly prevalent, and dogs may participate in the transmission chain of *L. braziliensis*. **Supported by:**Instituto Nacional de Ciência e Tecnologia em Doenças Tropicais (INCT-DT), CNPq #465229/2014-0
Keywords: Canine tegumentary leishmaniasis;Leishmania braziliensis;Canine subclinical infection.

HP-32 - **Evaluation of the amount of ROS in *T. cruzi* intracellular multiplication**

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Upon infection with T. cruzi, host cells respond producing reactive oxygen species (ROS). Previous studies have shown that cardiomyocyte (CM) infection with T. cruzi JG strain (T. cruzi II) induces higher amounts of ROS, as well as higher amounts of intracellular parasites, when compared to those infected with T. cruzi Col1.7G2 strain (T. cruzi I). Additionally, inhibition of ROS was able to decrease the number of JG strain intracellular parasites, but not Col1.7G2. These results together suggested that, at least to some T. cruzi strains, ROS production might be signaling to the parasite and contributing to increasing its intracellular multiplication rate. We have also shown that Col1.7G2 produces more antioxidant enzymes, suggesting that it may be less affected by an oxidative environment. Whether ROS induction of intracellular multiplication is related to the parasite strain or just the amount of ROS is still not known. To test this, we evaluated T. cruzi Y strain (T. cruzi II) intracellular multiplication after H9c2 cardiomyocyte pre-treatment with different concentrations of H2O2. Using CM-H2DCFDA, a fluorescent indicator of ROS production, we showed that cells treated with 15 or 30µM H2O2 presented 5- and 6-times higher amounts of ROS, respectively, when compared with the control, non-treated. We then infected H2O2 pre-treated cells and evaluated parasite intracellular multiplication 48h and 72h post parasite exposure. At 48 hours, the number of intracellular parasites was higher in cells treated with 30 µM H2O2, when compared to cells treated with 15µM, being both higher than in non-treated cells. At 72h, the number of intracellular parasites was higher in cells treated with 15µM H2O2, when compared to 30µM H2O2 treated and non-treated cells. For the latter, the number of parasites seems to stagnate. This may indicate that the amount of ROS play a major role in parasite intracellular multiplication.
Keywords:T; cruzi;reactive oxygen species;intracellular multiplication.

HP-33 - **Evaluation of the susceptibility of Brazilian isolates of *Toxoplasma gondii* to drugs used in the treatment of toxoplasmosis**

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Toxoplasma gondii, the etiological agent of toxoplasmosis, is an obligate intracellular protozoan. Brazil has a great diversity of recombinant or unusual strains, which gives the species a high genetic variability in the country, which differ considerably from those that circulate in Europe and North America. Little is known about the susceptibility profile of Brazilian strains in the face of conventional chemotherapy (Reynolds et al., 2001; Doliwa et al., 2013). Thus, the objective of this study was to evaluate the effectiveness of the drugs pyrimethamine and sulfadiazine. Conventionally used for treatment in humans, to control the infection of three atypical strains of *T. gondii* (TgCTBr12, TgCTBr14 and TgCTBr18), isolated from congenitally infected children in in vitro assays. In vitro susceptibility assays showed that pyrimethamine at concentrations of 0.25 µM, 0.5 µM, 1 µM and 2 µM helped to reduce parasite proliferation in the three studied strains. The IC50 value (mean inhibitory concentration) of pyrimethamine observed for the TgCTBr14 strain was 0.1469 µM, for the TgCTBr18 strain it was 0.06209 µM and for the TgCTBr12 strain it was 0.02913 µM. However, the strains studied are not susceptible to the different sulfadiazine doses tested (125 µM, 250 µM and 500 µM). Of the conventionally used drugs, pyrimethamine can be used to treat infection by the TgCTBr12, TgCTBr14 and TgCTBr18 strains of T. gondii. Furthermore, it would be interesting to evaluate the effectiveness of the association between these two drugs at different concentrations. New studies, which evaluate a treatment time greater than 48 hours, also need to be carried out, to confirm the lack of efficacy of sulfadiazine. **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.

HP-34 - **NEUTROPHIL ELASTASE AND CATHEPSIN G CONTRIBUTE TO MACROPHAGE INFECTION BY Leishmania amazonensis**

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Leishmania amazonesis depends on the production of type I interferons to establish an infection in macrophages. Our group has shown that the activity of a host serine peptidase, neutrophil elastase (NE), leads to the activation of a pathway involving Toll-like receptor 4 (TLR4) upon Leishmania spp. infection in macrophages, resulting in the production of IFN-β from these cells. While NE and IFN-β are detrimental to *L. major*, *L.donovani* requires NE activity and the resulting IFN-β for efficient growth in macrophages. We therefore asked whether *L. amazonensis* was, likewise, dependent on the components of this pathway by investigating parasite internalisation by macrophages (at 3 hours). We found that parasites were internalised significantly less by macrophages from NE-deficient mice than those from the genetic background strain. A similar profile was observed when TLR4 was inhibited prior to *L. amazonensis* infection. Taking this further, we investigated the involvement of another host serine peptidase of the same family, cathepsin G (CG), in the phagocytosis of *L.amazonensis*. As was seen in the absence of NE, when CG was inhibited prior to infection, *L. amazonensis* was internalised at a lower rate. Overall, we show that *L. amazonensis* requires both NE and CG for the initial stage of host cell infection, and that uptake of *L. amazonensis* is dependent on TLR4. **Supported by:**FAPERJ; CNPq **Keywords:**Leishmania;Elastase Neutrofílica;TRL4.

HP-35 - *Leishmania* spp. expressing red fluorescence (*tdTomato*) as model for screening of anti-*Leishmania* compounds

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The main challenges associated with leishmaniasis chemotherapy are drug toxicity, the possible emergence of resistant parasites, and a limited choice of therapeutic agents. Therefore, new drugs and assays to screen and detect novel active compounds against leishmaniasis are urgently needed. We thus validated *L. braziliensis* and *L. infantum* that constitutively express the tandem tomato red fluorescent protein *(tdTomato*) as a model for large-scale screens of anti-*Leishmania* compounds. Confocal microscopy of *Lb* and *Li::tdTomato* revealed red fluorescence distributed throughout the entire parasite, including the flagellum, and flow cytometry confirmed that the parasites emitted intense fluorescence. We evaluated the infectivity of cloned promastigotes and amastigotes constitutively expressing tdTomato, their growth profiles in THP-1 macrophages, and susceptibility to trivalent antimony, amphotericin (AmB), and miltefosine *in vitro*. The phenotypes of mutant and wild-type parasites were similar, indicating that the constitutive expression of *tdTomato* did not interfere with the evaluated parameters. We applied our validated model to a repositioning strategy and assessed the susceptibility of the parasites to eight commercially available drugs. We also used our new model to screen 32 natural plant and fungal extracts and 10 pure substances to reveal new active compounds. The mutant parasites expressing *tdTomato* could serve as a model for assays to evaluate the susceptibility of anti-*Leishmania* compounds in vitro. The infectivity of mutant and wild-type parasites in BALB/c mice were similar. Standardizing our methodology would offer more rapid, less expensive, and easier screens of compounds against *L. braziliensis* and *L. infantum in vitro* and *in vivo*. Our method could also enhance the discovery of active compounds for treating leishmaniasis.
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Keywords: Leishmania;compound screening;chemotherapy.

HP-36 - Infection of non-phagocytic cells by promastigotes and amastigotes of Leishmania amazonensis: a comparative study of host cell invasion and the cellular mechanisms involved in cell entry

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Intracellular parasites are characterized by the need to invade and stablish intracellular cycle within host cells. For *Leishmania* spp., the causative agents of Leishmaniasis, it is assumed that the parasites are phagocytosed and live inside professional phagocytic cells. However, the presence of *Leishmania* spp. amastigotes within non-phagocytic cells have been described for decades in animal and human lesions. Although the parasites of the genus *Leishmania* have been known for a long time, we know very little about how they can infect non-phagocytic cells and the possible role of these cells in the biology of the parasite. The objective of this work was to characterize and compare the mechanisms involved in the invasion of non-phagocytic cells by promastigotes and amastigotes of *Leishmania*, both highly infective to mammalian hosts. We developed an infection model using mouse embryonic fibroblasts (MEF) and amastigotes and promastigotes of *Leishmania amazonensis*. We performed time-course infection assays, labelled infected cells and parasites to visualize molecules, organelles and structures possibly involved in cell invasion. Our results show that while promastigotes can invade non-phagocytic cells independently of host cell cytoskeleton, amastigotes are highly dependent on F-actin polymerization, being able to strongly induce this process to promote cell invasion. By comparing the infection rates in time-course experiments it was also possible to conclude that amastigotes are more invasive for non-phagocytes than promastigotes. Thus, our results suggest that the two evolutive forms of *Leishmania* spp. have different mechanisms to invade non-phagocytic cells and that amastigotes, the form of the parasite responsible for infection amplification in the mammalian host, are particularly infective to non-phagocytes.
Supported by:CAPES **Keywords:**Leishmania spp;;Cell Invasion;Non-phagocytic Cells.

HP-37 - **Assessing the infectivity of *Leishmania infantum* promastigotes to non-phagocytic cells using HeLa, C2C12 and Mouse Embryonic Fibroblasts as host cell models.**

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Intracellular parasites are characterized by the need to invade and establish an intracellular cycle within host cells. For *Leishmania* spp., the causative agents of Leishmaniasis, it is assumed that the parasites are phagocytosed and live inside professional phagocytic cells, notably macrophages. However, the presence of *Leishmania* spp. amastigotes within non-phagocytic cells have been described for decades in animal and human lesions. The presence of *Leishmania* spp. amastigotes in non-phagocytic cells is an overlooked aspect of the biology of these parasites that may contain key elements to understand unsolved gaps of their biology such as parasite persistence and drug resistance, since parasites may be out of reach of drugs and immune effectors while living within these cells. For *Leishmania amazonensis*, the causative agent of human cutaneous leishmaniasis, we have recently demonstrated that promastigotes are able to infect and persist within fibroblasts in which they enter using host cell lysosomes, thus by a non-phagocytic route. Here our objective was to assess the infectiveness of *Leishmania infantum*, a causative agent of human visceral leishmaniasis, to three models of non-phagocytic cells: HeLa, C2C12 and Mouse Embryonic Fibroblasts (MEF). First, we produced GFP-expressing promastigotes of *L. infantum* and compared parasite growth to show that both WT and GFP-expressing promastigotes displayed the same growth pattern. In the sequence, we cultivated HeLa, C2C12 and MEF and performed infection assays using GFP-expressing *L.infantum* promastigotes. After infection, cells were labelled using phalloidin and anti-LAMP1 antibodies which showed that parasites were internalized and established themselves as intracellular amastigotes living within typical intracellular vacuoles rich in lysosomal markers, as expected for *Leishmania* spp. **Supported by:**FAPEMIG **Keywords:**Leishmania infantum;Parasite Life Cycle;Cell Invasion.

HP-38 - **Infection of muscle, epithelial and connective cells by *Leishmania amazonensis*: assessing parasite viability and the potential role of non-phagocytic cells as parasite reservoir**

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Intracellular parasites are pathogens that need to invade and exert at least part of their life cycles within host cells. For *Leishmania spp*. it is assumed that the parasite is phagocytosed and lives in professional phagocytic cells. However, several works have described in vivo the presence of intracellular stages *Leishmania spp*. in non-phagocytic cells such as muscle, epithelial and connective cells.The presence of *Leishmania spp*. amastigotes within non-phagocytic cells is an overlooked aspect of their biology that may provide important knowledge to understand unsolved gaps such as drug resistance, parasite persistence and late infection reactivation. Our objective with this work was to study the infection of muscle, epithelial and connective cells by *Leishmania amazonensis*, to assess the viability of the parasites inside these cells and evaluate their ability to function as parasite reservoir, ultimately leading to the infection of macrophages. We used light, conventional and confocal microscopies, immunobiochemical methods and flow cytometry to assess *L. amazonensis* infectivity in muscle fibers,epithelial cells and fibroblasts as long as the ability of these cells in producing leishmanicidal effectors such as nitric oxide (NO) and reactive oxigen species (ROS). Our results show that both amastigotes and promastigotes can invade and persist within these cells as typical intracellular amastigotes and that,unlike macrophages,they are unable to produce NO or ROS, thus facilitating parasite persistence. When macrophage-like cells (RAW cells) were co-cultured with the infected non-phagocytic cells, infection spread to the macrophages, demonstrating that parasites could be transferred from one cell to the other–similar to the previously preconized for neutrophils and named as the “Trojan Horse Hypothesis”. Our studies suggest that non-phagocytic cells can function as parasite reservoirs from which the amastigotes can reach the macrophages.
Supported by:CNPq **Keywords:**Leishmania spp;;Cell invasion;Non-phagocytic cells.

HP-39 - **Characterization of Nfs, Isd11 and Mtu1: role in the mitochondrial thiolation pathway of tRNAs in *Trypanosoma cruzi***

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One of the key biological features of *Trypanosoma cruzi* is the presence of a single and branched mitochondrion, which harbors the mitochondrial genome (kDNA) in a structure called kinetoplast. The kDNA does not encode for tRNAs, which are imported from the cytoplasm in order to allow mitochondrial protein expression. tRNAs are extensively post-transcriptionally modified, and among those modifications identified so far, thiolation can be found at uridines at positions 33 and 34 of tRNAs. Remarkably, uridine thiolation at position 33 (sU^{33}) has only been identified in trypanosomatids. It has been shown that sU^{33} negatively modulates $tRNA^{Trp}$ editing in the mitochondria of *Trypanosoma brucei*, which directly affects mitochondrial gene expression. The mitochondrial thiolation of tRNAs requires the protein complex formed by the cysteine desulfurase (Nfs; EC:2.8.1.7), a tRNA-specific 2-thiouridylase (Mtu1; E.C.:2.8.1.14) and an accessory protein (Isd11), besides of using the amino acid cysteine as a thiol group donor. The *T. cruzi* genome encodes putative orthologs of genes involved in this pathway. Aiming to investigate the impact of these enzymes on the biology of *T. cruzi,* hemi- and total-knockout strains for the genes putatively involved in the mitochondrial thiolation pathway were generated using CRISPR-Cas9. Phenotypical characterization of $Isd11^{-/+}$ and $Mtu1^{-/-}$ cell lines demonstrated no impact in cell proliferation. However, in $Isd11^{-/+}$ mutants, differentiation of epimastigotes to the metacyclic trypomastigote are 2.6 times more than the control. Additionally, the putative coding sequences of Nfs, Isd11 and Mtu1 were endogenously tagged with mNeonGreen and c-myc tags to allow subcellular localization of these proteins. So far, C-terminus tagging of Nfs showed mitochondrial localization. In *T. cruzi*, little is known about the functioning of the tRNA thiolation pathway, so this study may elucidate the link between amino acid metabolism and regulation of gene expression.
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Keywords: Trypanosoma cruzi;CRISPR-Cas9;tRNA.

HP-40 - **IN VITRO INFECTION OF PERITONEAL MACROPHAGES WITH Leishmania braziliensis PROMASTIGOTES OR AMASTIGOTES FROM IFN-GAMA KNOCKOUT MICE LESION**

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INTRODUCTION: Leishmania parasites infect the host as promastigotes after sandfly bite, but internally, amastigotes maintain the infection of macrophages during disease development. **OBJECTIVE:** Compare the susceptibility of Thioglycolate-elicited peritoneal macrophages to stationary phase promastigotes or amastigotes from mice lesion. **METHODS**: *Leishmania braziliensis (Lb)* parasites were isolated from the hind paw of infected IFNg Knockout mice or stationary phase promastigote from culture to infect peritoneal macrophages from thioglycolate-elicited BALB/c mice to evaluate parasite burden (PB) defined as percentage infected cells (%IC) parasites per infected macrophage (P/C) and infection index (II) 3h, 72h, 144h and 216h post infection (PI). **RESULTS:** There was a significant decrease in PB in infections with stationary phase promastigotes (SPP) from 3h to 144h PI (P/C = 3,5±0,3 to 2,3±0,1; II= 223,0±58,4 to 107,8±21,7 respectively). Differently, there was a maintenance of the PB in infections with amastigotes (LA) from 3h and throughout 72h and 144h (%IC = 76,2±5,4; 79,6±8,8; 79,0±5,4) (P/C = 4±0,8; 5±1,2; 5,4±1,1) (II = 315,7±91,3; 409,8±140,4; 431,9±117,8). The %IC was greater in infections with LA during 72h and 144h (%IC = 79,6±8,8; 79,0±5,4) when compared to infection with SPP (%IC = 50,8±5,3; 45,8±5,7). Interestingly there was a sudden decrease of infected macrophages in infection with LA from 144h to 216h (44,6±7,7%) matching the %CI with SPP at 216h PI (44,4±5%). **CONCLUSION:** LA from mice lesion are more adapted to survive inside BALB/c macrophage than SPP.
Supported by:INCT/MCTI/CNPQ/Universal 14/2014 **Keywords:**Leishmaniasis;mice;Parasite burden.

HP-41 - **Removal of Complement Membrane Attack Complex by Plasma Membrane Repair in *Leishmania amazonensis:* an ancestral cellular mechanism of Eukaryotes promoting pathogen immune escape.**

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Plasma membrane repair (PMR) is a key process present in eukaryotic cells that promotes the resealing of damages eventually inflicted to the plasma membrane (PM). Cells under constant mechanical stress, such as muscle cells, or cells attacked by bacterial pore-forming proteins (PFP) can use this mechanism to prevent lytic death. In the case of attack by PFPs, cells can trigger PMR to remove the pores from their PMs. PMR triggered by PFPs typically involves 1-Ca^{2+} influx; 2- lysosomal exocytosis and 3- pore removal by either endocytosis or membrane budding. The Complement System is one of the first shields of innate immunity of hosts against pathogens. Given that the Membrane Attack Complex (MAC), which is the result of the final activation of the Complement System, is a lytic transmembrane pore,we postulated that mammalian cells and eukaryotic pathogens can use PMR to avoid self-lysis and to evade the immune system, respectively. By using conventional, confocal and live imaging microscopies, immunobiochemical methods and flowcytometry we demonstrated that mammalian cells are able to repair MAC pores by triggering typical PMR responses. Furthermore, and given that the major function of MAC is to pierce the membrane of pathogens, we hypothesized that PMR could be used by eukaryotic parasites, such as *Leishmania* spp.to evade the Complement System by removing MAC pores eventually formed on their PMs. Using fresh human complement and *Leishmania amazonensis* promastigotes we were able to show that PMR is, indeed, conserved in this parasite and that, like mammalian cells, *L.amazonensis* promastigotes can resist complement killing by removing MAC lytic pores from their membranes. Thus, our results show that PMR is evolutionarily preserved in *Leishmania* spp. and that - this physiological mechanism can be used by parasites as an extra weapon to evade the final activation of the Complement System and to escape host's first line of defense. **Supported by:**CNPq,
Keywords: Leishmania amazonensis;Plasma Membrane Repair;Immune evasion.

HP-42 - **In silico virtual screening to discovery new natural compound for visceral leishmaniasis chemotherapy**

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Treatment for leishmaniasis is still a challenge so is necessary to find orally compounds that are less toxic and more effective. Drug discovery has been relied on the assistance of computational tools, a promising approach to tackle this problem. In this context, the objective of this project was to select natural compounds for a new chemical entity using computational approach based on *L. infantum* trypanothione reductase (TrLi). First, a library with 67 natural products was created, and ADMET parameters was estimated by pkCSM server to identify safe compounds for oral administration. 4 of them fill this criteria and through virtual screening PyRX software, the ΔG value was calculated, ranging from -7.2 to -9.4 kcal/mol, indicating that they interact at the TrLi active site. NC3 presented ΔG values of -9.0 and -8.9 kcal/mol for the oxidized and reduced forms of TrLi, respectively. Calculations performed by Autodock Tools software estimated that NC3 showed an estimated ΔG and Ki values of -7.9 kcal/mol and 1.7 μM for oxidized LiTr, and -8.31 kcal/mol and 805 nM for reduced LiTr, and interacts with residues of the catalytic site. Next, *L. infantum* promastigotes were incubated with different concentrations of NC3 (0.97-2000 μM) for 72 hours, the viable cells were estimated fluorometrically by resazurin and ROS generation by H2DCFDA. NC3 inhibited 98% of the parasites in a dose-dependent manner with an IC50 of 194.3 μM and accumulated intracellular ROS level in 3.7 fold related with non-treated group at the highest concentration used (500 μM) with a linear correlation (R2 = 0.92). Finally, a citoxicity assay demonstrated a CC50 of 483.4 μM and an IC50 of 2.6 μM in intracellular amastigote with a selectivity index of 186. Together our data demonstrate that the structure-based virtual screening is an excellent ally for drug discovery, and that compound NC3 is a potential competitive inhibitor of trypanothione reductase and candidate for the treatment of leishmaniasis. **Supported by:**FAPERJ; CNPQ; IOC/FIOCRUZ
Keywords: Structure-based virtual screening;Trypanothione reductase;Natural products.

HP-43 - **Resident and Stimulated Peritoneal Macrophages from mice control proliferation of *Toxoplasma gondii* strain ME-49 in iNOS+ and iNOS- populations**

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Toxoplasma gondii is an obligate intracellular parasite of homeothermic vertebrates. This parasite infects nucleated cells including macrophages. Macrophages control proliferation of the parasite through the production of nitric oxide (NO) produced by inducible NO synthase (iNOS). Virulent *T. gondii* strains inhibit NO production and can modulate iNOS expression depending on the type of macrophage. The aim of this work was to evaluate whether *T. gondii* of the ME-49 strain (less virulent) inhibits NO production by modulating iNOS expression in resident peritoneal macrophages (ResMO) and stimulated peritoneal macrophages (StdMO) obtained after peritonitis caused by inoculum of *T. gondii* tachyzoites. Macrophages were obtained by peritoneal lavage, cultured and activated with interferon-ɣ and lipopolysaccharide for 24h. After activation, they were infected with a 5:1 parasite:macrophage ratio, the production of NO was evaluated using the Griess reagent and iNOS expression was quantitatively and qualitatively evaluated by fluorescence microscopy. ResMO produced around 17µM and StdMO produced about 35µM of NO. Difference of NO production was not detected after infection of both types of macrophages. By fluorescence microscopy it was observed that after 24h of interaction, iNOS+ infected ResMO increased from 30% to 48%. Infectivity index (IF) dropped from 78.9 to 3.9 in iNOS+ population while ResMO iNOS- dropped from 85.0 to 8.4. After 24h of interaction, StdMO infected iNOS+ changed from 93% to 52% and the IF of the iNOS+ population dropped from 167.8 to 22.8 and in the iNOS- population from 157.1 to 32.2. It is concluded that in 24 hours of interaction, ResMO and StdMO did not change the production of NO. iNOS expression increased in ResMO and decreased in StdMO, but both controlled parasite replication in iNOS+ and iNOS- populations, indicating that this control is independent of iNOS expression. **Supported by:**FAPERJ-UENF **Keywords:**Toxoplasma gondii;Macrófagos;iNOS.

HP-44 - **Modulation of infection by *Toxoplasma gondii* strain ME-49 in M1 macrophages cultivated in collagen I biofilm when compared to rigid substrate**

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Conventional cell culture is performed on rigid substrates (SR). However, alternatives using three dimensional substrates are on the rise. Collagen I biofilm (COL I) is an alternative of great importance because collagen is an abundant extracellular matrix protein related to cell growth, motility and regulation. Macrophages are classified according to their activation profile. M2 macrophages induce humoral response and maintain tissue homeostasis. M1 macrophages are more microbicidal, capable of killing intracellular pathogens by high production of reactive oxygen/nitrogen species like nitric oxide (NO). NO acts on the site of infection and reduces the proliferation of parasites such as *Toxoplasma gondii*, which causes Toxoplasmosis, a disease spread worldwide. *T. gondii* grows in host cell in the form of tachyzoites inside parasitophorous vacuoles inducing the acute phase of the disease. We compared NO production and prevalence of infection with *T. gondii* of the ME-49 strain in activated M1 macrophages cultured in COL I and SR. COL I was obtained from rat tail. Raw 264.7 macrophages were cultured on COL I and SR, were M1 activated for 24 h with lipopolysaccharide and interferon-y, and infected for 24 h with a 1:1 macrophage:tachyzoite ratio. NO was evaluated by the Griess reaction and infection measured by direct count of cells after Giemsa staining. M1 macrophages cultured in COL I produced less NO compared to M1 macrophages cultured in SR. After 24 h of infection, few tachyzoites were seen in the M1 macrophages cultured in COL I, but M1 macrophages cultured in SR controlled the infection, presenting vacuoles without tachyzoites. Thus, M1 macrophages cultivated in COL I are less microbicidal. COL I can be used as a key alternative substrate to provide a possible more realistic response of the immune system's functionality and may constituent an interesting culture substrate to study the interaction of host cells with pathogens.
Supported by:FAPERJ, CAPES **Keywords:**Collagen ;Macrophage;Toxoplasma gondii.

HP-45 - **ANTAGONIC ROLES OF THE NEUTROPHIL ELASTASE-TLR4 PATHWAY AND CATHEPSIN G IN EXPERIMENTAL INFECTION BY** *L. braziliensis*

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Leishmania sp. have genes similar to bacterial ecotins, potent inhibitors of trypsin-type serine peptidases (SPs), which were thus named ISPs. ISP2 targets neutrophil elastase (NE) in macrophages preventing TLR4 activation during phagocytosis. The NE-TLR4 pathway results in the production of type I interferons, which are leishmanicidal for *L. major* but required for successful *L. donovani* infection. Here, we addressed the role of host SPs in the infection by *L. braziliensis*. Internalisation of *L. braziliensis* by murine macrophages was reduced by NE inhibition or in macrophages from NE-knockout mice (*ela2*$^{-/-}$). In mice, NE inhibition led to reduced plasma infiltration in the footpad 3 h post-infection, accompanied by reduced parasite burdens in the lymph nodes at week 2, as compared to untreated infected controls. Macrophage infection was reduced following the use of a TLR4 inhibitor or an antagonist to PAR2, a receptor that crosstalks with TLR4, while inhibition of TLR4 had no effect in the infection of *ela2*$^{-/-}$ macrophages, indicating that NE acts through TLR4. BALB/c mice infected with *L. braziliensis* in the presence of the PAR2 antagonist showed reduced plasma infiltration in the footpad at 24 h, followed by reduced parasite loads in the lymph nodes at week 5. We further addressed the contribution of the trypsin-type SP, cathepsin G (CG). Infection of macrophages derived from CG-knockout mice or control macrophages treated with a CG inhibitor enhanced parasite uptake. CG inhibition augmented infection of *ela2*$^{-/-}$ macrophages, suggesting that NE and CG modulate phagocytosis of *L. braziliensis* through opposite and independent pathways. CG-knockout mice displayed increased footpad lesions as compared to infected 129Sv mice, while parasite burdens were similar at week 5. Taken together, our findings indicate that the NE-TLR4 pathway acts positively in parasite uptake by macrophages and in the establishment of infection at distant sites, while CG exerts a protective role. **Supported by:**FAPERJ, CNPq and UK research abd Innovation **Keywords:**Leishmania braziliensis;Serine Peptidases;ELA-TLR4 pathway.

HP-46 - **Immunosuppression post-heart transplantation promotes a decrease in miRNA-146b-5p levels in the blood of patients with Chagas disease**

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The most severe form of Chagas disease (CD) is chronic Chagas cardiomyopathy (CCC) which, in its last stage, has heart transplantation as its main outcome. Post-transplant CD reactivation occurs due to treatment with immunosuppressants, which are necessary to prevent graft rejection. Previously, miR-146b-5p was shown to be related to the inflammatory process triggered by *T. cruzi* and the production of cytokines, being a potential biomarker of tissue damage and cardiotoxicity in CCC. . There are few studies of biomarkers for monitoring CD reactivation. Thus, in this study, we analyzed miR-146b-5p levels in the blood of transplanted and immunosuppressed CD patients, with or without treatment with benznidazole (Bz). miR-146b-5p levels in the blood of transplanted and immunosuppressed CD patients with or without Bz treatment. The extraction of miRNAs was performed in samples from patients with positive serology for CD, positive or negative for the presence of *T. cruzi* in the blood (by qPCR), after undergoing heart transplantation and immunosuppression. As a control, samples from patients with negative serology for CD were used. Then, reverse transcription and gene expression analysis was performed for target miR-146b-5p and cel-miR-39-3p, as an exogenous internal control of RT-qPCR. The control group, transplanted patients, but with negative qPCR for *T. cruzi,* after treatment with bz), showed a significant increase in the expression of miR-146b-5p (2.5 times; $p<0.05$). Transplanted patients, but with positive qPCR for *T. cruzi,* showed a significant decrease in the expression of miR-146b-5p (6 times; $p<0.01$) compared to patients with negative qPCR for *T. cruzi.* This suggests that miR-146b-5p has the potential to be studied as a biomarker of reactivation by immunosuppression in CD, being important for early detection of reactivation and indication for treatment.
Keywords: Immunosuppression;Cardiac Transplantation;MicroRNAs.

HP-47 - **Cellular and molecular mechanisms involved in the formation and detachment of the parasitophorous vacuole during infection of non-phagocytic cells by** *Leishmania amazonensis*

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Intracellular parasites are characterized by their need to invade and exert at least part of their cycle within host cells. These pathogens present a series of strategies to overcome the barrier imposed by the plasma membrane and enter their host cells. For *Leishmania* spp. it is assumed that the parasite is phagocytosed and lives inside phagocytes. However, several studies have shown the presence of these parasites inside non-phagocytic cells. Despite being able to establish infection after capture by phagocytosis, it has been shown that promastigotes of *Leishmania amazonensis* are also capable of inducing their penetration into host cells via lysosome sequestration and subversion of the cellular process of plasma membrane repair. Regardless the form of penetration employed, a fundamental step during invasion is the fission of the plasma membrane at the invasion site, culminating with the detachment of the nascent vacuole and its release into the host cell. Despite efforts to discover the molecular effectors involved in the process and some evidence pointing to the membrane fission proteins dynamin and endophilin, the process is still unknown. In this work we investigate the molecular machinery involved in the initial stages of biogenesis of the parasitophorous vacuole of intracellular parasitic trypanosomatids using *L.amazonensis* promastigotes and mouse murine fibroblasts (MEFs) as a model. Our hypothesis is that proteins of the ESCRT complex (endosomal sorting complex required for transport), classically promoters of membrane scission, may be involved in cell invasion by these parasites. In this project we are silencing the expression of some ESCRT complex proteins in MEFs, proceeding infection assays using *L.amazonensis* promastigotes and quantifying infections. To verify if ESCRT complex proteins colocalize with the nascent parasitophore vacuole, we are proceeding time-course infections and performing immunofluorescence and fluorescence microscopy analyses. **Supported by:**FAPEMIG
Keywords: Leishmania spp;;Cell Invasion;Membrane scission.

HP-48 - **Preservation of blood in guanidine-EDTA solution for the analysis of microRNAs in patients with Chagas disease**

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Chagas disease (CD) is a neglected tropical disease caused by the flagellate protozoan *Trypanosoma cruzi*. The gold standard for evaluating the therapeutic efficacy of CD is the seroconversion of conventional serological tests, which can take years to be achieved. Thus, the development of biomarkers of therapeutic efficacy in Chagas disease is the subject of intense research. MicroRNAs (miRNAs) are a class of small, single-stranded, non-coding RNAs that act to regulate the expression of target messenger RNAs. In a previous study by our group, 641 miRNAs from mice infected with *T. cruzi*, treated or not with benznidazole in the chronic phase of the disease, were analyzed, highlighting 20 miRNAs that increased or decreased expression in infected animals, returning to normal levels after the treatment. Considering that many blood samples are preserved in a 6M guanidine hydrochloride-EDTA 0.2 M pH 8.0 solution, we evaluated the ability of this solution to preserve miRNAs when mixed with human blood in a 1:1 ratio. For this, blood samples were stored with and without guanidine, in parallel, and were artificially contaminated with two exogenous *C. elegans* miRNAs (cel-mir39 and cel-mir54). Expression analysis of exogenous miRNAs and human microRNA miR-146b-5p and small U6 RNA was performed by the comparative Ct method, using pre-validated TaqMan assays (Applied Biosystems). We observed that blood samples preserved in guanidine showed Ct values even lower than samples without the preservative agent, both for endogenous and exogenous miRNAs. This result paves the way for the use of many retrospective samples from clinical studies in Chagas disease, which are being preserved in guanidine-EDTA only for *T. cruzi* DNA analysis. In view of this, the analysis of miRNAs in blood with guanidine-EDTA can be promising in studies seeking biomarkers of pathogenesis and therapeutic response in Chagas disease.
Keywords:microRNAs;Guanidina-EDTA;Doença de Chagas.

HP-49 - **Exploring the Shikimate Metabolic Pathway in *Plasmodium falciparum*: Uncovering Key Insights**

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Malaria is a disease that's impacting the tropical regions, caused by protozoan parasites belonging to the phylum Apicomplexa, specifically the Plasmodium genus. Among those, Plasmodium falciparum stands out as the causative agent of severe cases. The emergence of drug resistance in different endemic regions demands the identification of novel targets for effective disease treatment, while minimizing host impact. The intriguing observation of glyphosate, a widely used herbicide, inhibiting the proliferation of P. falciparum raises questions regarding the presence of the shikimate pathway on those parasites. Although this metabolic pathway has been well-established in plants, bacteria, and fungi, its absence in mammals renders an appealing target for the development of new antimalarial drugs. The shikimate pathway comprises seven enzymatic steps leading to the synthesis of chorismate, a pivotal metabolite involved in diverse biochemical processes, including the biosynthesis of folates and prenylquinones. To explore the shikimate pathway in P. falciparum, we conducted experiments using parasite cultures or C3A cells (HepG2/C3A) cultivating them under compound concentration to determine the 50% inhibitory concentration, thereby assessing parasite proliferation. By investigating the impact of these compounds on parasite growth, our study aims to shed light on the potential of targeting the shikimate pathway for the development of novel antimalarial drugs. Inhibition tests showed a decrease in the proliferation of parasites against the compounds used, mainly chlorogenic acid and its derivatives, additionally, these compounds did not exhibit cytotoxicity in C3A cells (HepG2/C3A) in tested concentrations. After looking over the results obtained, we concluded the tested compounds are capable of inhibiting the proliferation of P. falciparum cultures, against to what was observed in mammalian cells. **Supported by:**FAPESP 2019/08637-5 **Keywords:**Malaria;Plasmodium falciparum;Shikimate pathway.

HP-50 - **Host cell cholesterol and T. cruzi intracellular cell cycle**

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Trypanosoma cruzi is a protozoan parasite and the etiological agent of Chagas disease, *T. cruzi* subverts a membrane repair mechanism, dependent on host cell lysosomes, for cell invasion. Data from our group has shown that cells lacking a lysosomal protein, LAMP (LAMP1/2$^{-/-}$ fibroblasts), are less permissive to invasion, but more susceptible to parasite multiplication. Impaired invasion was shown to be due to cholesterol trafficking disruption, which leads to free cholesterol accumulation in the cell interior, compromising membrane repair process. Previous data from our group suggested host cell cholesterol was also important for *T. cruzi* intracellular multiplication. Treatment of wild type fibroblasts (WT) with atorvastatin was shown to increase intracellular parasite multiplication. Atorvastatin inhibits intracellular cholesterol synthesis. On the other hand, it is also known to enhance the expression levels of LDL receptor, leading to increased cholesterol uptake. In order to confirm the influence of cholesterol in *T. cruzi* intracellular life cycle, in the present work we evaluated the effects of serum deprivation in parasite intracellular multiplication. As for atorvastatin treatment, serum deprivation also leads to increase in intracellular cholesterol, but in this case by enhancing endogenous cholesterol synthesis. For this, we submitted WT and LAMP1/2$^{-/-}$ fibroblasts to treatment with atorvastatin or serum deprivation for 6h, previous to infection with Dm28c strain of *T. cruzi.* After parasite exposure, cells were washed and the number of intracellular parasites, at 24, 48 and 72 hours post-infection was evaluated. As expected, serum reduction as atorvastatin treatment increased the number of intracellular parasites in WT cells. Both treatments, on the other hand, did not alter parasite multiplication in LAMP1/2$^{-/-}$ cells. Likely due to the fact that the latter already have an increased content of intracellular cholesterol. **Supported by:**CAPES
Keywords:T; cruzi;Cholesterol;LAMP.

HP-51 - **Evaluation of the efficacy anti-*Leishmania amazonensis* the drug buparvaquone with a ubiquinone biosynthesis inhibitor using *in vitro* model.**

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Leishmaniasis is an important problem in public health caused by protozoa of the genus *Leishmania*. These parasite presents two evolutionary forms: promastigote (extracellular) that is present in the insect vector and intracellular amastigote form that resides mainly in macrophages, present in the vertebrate host. The treatment of this disease is considered a major challenge, due to the limited therapeutic arsenal and high toxicity of drugs. To study the activity of a drug for this disease, it is necessary that it acts mainly in the intracellular amastigote form, and this makes the search for new drugs very difficult, since it requires macrophages to maintain the intracellular amastigote. So, it is necessary to present activity in intracellular amastigotes forms for the study to be more thorough and have greater relevance, thus being able to continue the study in the *in vivo* model. This parasite is distinguished from mammalian cells by having a unique and essential mitochondrion, which makes it an important therapeutic target. In the inner membrane of mitochondria, ubiquinone is found, an isoprenoid that plays an important role in the electron transport chain. Based on this information, the drug buparvaquone was chosen, which is an analogue of ubiquinone, and already has anti-*Leishmania* activity described in the literature. An inhibitor of ubiquinone biosynthesis, 4-iodobenzoate (4-IO) was chosen to be studied in association with buparvaquone, which showed activity only in the promastigotes forms (unpublished). The results of the therapeutic association showed an additive effect on the promastigotes forms. As for the intracellular amastigotes, the results showed that when testing buparvaquone with fixed and non-toxic concentrations of 4-IO, the effect of buparvaquone was potentiated two to three times. The results obtained so far encourage us to continue studies of the mechanism of action, as well as to carry out studies using the murine model. **Supported by:**Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) - número processo 2021/01877-0
Keywords: Leishmaniasis;therapeutic target;ubiquinone biosynthesis.

HP-52 - *In vitro* **Chemotherapy against *Leishmania amazonensis* promastigotes using novel metallocomplex compounds (AP1022)**

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Leishmaniases are diseases with high incidence and wide geographical distribution. They also have a wide spectrum of clinical manifestations related to the species of parasite that causes the disease. Among these clinical manifestations are local skin lesions caused by the species *Leishmania amazonensis.* The therapy available for this disease is based on the use of Amphotericin B or pentavalent antimonial drugs, which have side effects and drug-resistant strains have been reported. Therefore, it is necessary to develop new therapies to treat the infectious process. Metallocomplexes are composed of transition metals, which can be coordinated with drugs, and present possibilities of coordination and geometries with the ligand. This coordination allows the interact with the specific molecular target, mainly with biological molecules. Metallocomplexes may be an interesting alternative for use in chemotherapy of *Leishmania spp. in vitro*, as it was recently found for a cobalt metallocomplex against *L. amazonensis in vitro*, altering the parasite's flegellar pocket, leading to death by autophagy. In this work, we evaluated the *in vitro* effect of the compound AP1022 on the growth of promastigotes of *L. amazonensis* (strain WHOM/ BR/ 75/ Josefa). The parasites were treated with the compound in concentrations that varied from 1 to 100 µM and the growth after the treatment was quantified. To evaluate the effect of this metallocomplex cell viability of the LLC-MK2 host cell and an antiproliferative assay with promastigote of *L. amazonensis in vitro* were performed. The AP1022 compound showed an IC50 value of 0.98 µM to 1.80 µM after 3 and 5 days of treatment of promastigotes; no toxicity was found to the host cells. The results open a gateway to innovation, as the IC50 value indicated that the AP1022 complex as a drug capable of controlling the growth of the parasite in the promastigote form maintained *in vitro*. **Supported by:**CAPES
Keywords: Metallocomplexes;Leishmania amazonensis;Chemotherapy.

HP-53 - **Leishmania amazonensis extracellular vesicles (EVs) induce neutrophil extracellular traps (NETs).**

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Sandfly vectors inoculate *Leishmania* in a pool of blood together with promastigotes-derived extracellular vesicles (EVs). It has been shown that this co-injection induces inflammation and exacerbates leishmaniasis lesions. EVs are a heterogeneous group of particles released by cells that play a key role in intercellular communication, and carry proteins, RNA, DNA, lipids and metabolites, being easily transferred from one cell to another. Neutrophils, the major leukocyte in the blood, are one of the first cells to interact with the parasite. Upon interaction with *Leishmania*, these cells release neutrophil extracellular traps (NETs) that ensnare and are toxic to the parasite. Our investigation aimed to determine whether EVs can also induce NETs. Thus, we incubated neutrophils from healthy human blood with EVs secreted by promastigotes (150 nm by NTA) and measured the number of NETs produced by the picogreen assay. Our results showed that EVs induced NET release in a reactive oxygen species-dependent manner. NETs induced by EVs are inhibited by chloroamidine, BAPTA, inhibitors of myeloperoxidase (MPO), elastase, and gasdermin D. EVs stained by CA7AE and anti-GP63 are observed inside neutrophils and trapped in the NETs. Interestingly, EV-induced NET is specifically inhibited by phenanthroline. Together, our results ensure that ROS, elastase, MPO, peptidyl arginine deiminase, calcium and gasdermin D are involved in EVs-induced NET formation. **Supported by:**FAPERJ, CNPq, CAPES
Keywords: Leishmania;Extracellular Vesicles;Neutrophil Extracellular Traps (NETs).

HP-54 - **Unveiling the potential role of lncRNAs in the Murine Model of Cutaneous Leishmaniasis**

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Introduction: Cutaneous Leishmaniasis (CL) is characterized by ulcerative skin lesions that lead to significant and lasting scarring. Previous transcriptomics studies from human CL lesions caused by *Leishmania braziliensis* (*Lb*) have provided insights into critical aspects of immunopathology. To gain insights into the initial stages of CL infection, disease progression, and the healing process, we conducted comprehensive transcriptomic profiling of lesions and draining lymph nodes (dLNs) using a murine model that replicates *Lb*-induced CL in humans. **Methods:** BALB/C mice were infected with *Lb* in their ears, and ears and draining lymph nodes (dLN) were collected at the early (2, 6, and 48 hours, and 14 days) and late (35 and 77 days) phases post-infection. Three biological replicates per time-point were used for both tissues, and bulk RNA-seq was performed. CEMItool was used to identify gene modules (M) with significant co-expression during lesion development. **Results:** Gene co-expression analysis revealed dynamic changes shortly after the inoculation of *Lb* into the dermis, persisting until lesion healing. During the early phase, lesions exhibited the activation of modules associated with inflammation and wound healing. In the late phase, modules involved in Phagosome formation (M3) and LXR/RXR (M2) were significantly activated. Surprisingly, a distinct module (M4) containing 219 genes, including 63% unclassified genes and 23.3% long non-coding RNAs (lncRNAs), showed marked deactivated at Day 35. Correlations and enrichment analysis suggested the involvement of these lncRNAs in multiple pathways, particularly TCR and TLR receptor activation. **Conclusion:** The findings demonstrate a remarkable overlap of known pathways observed in both the murine model and human disease, emphasizing the translational relevance of the murine model. In addition, data presented in this study highlight new potential targets for therapeutic interventions in the treatment of Lb infections. **Supported by:**CAPES, PIAP/IGM/FIOCRUZ-BA (001/2017) **Keywords:**Cutaneous leishmaniasis;RNA seq;Inflammation.

HP-55 - **Effects of Adipose Mesenchymal Stromal Cells Extracellular vesicles in experimental infection from *Leishmania amazonensis***

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Leishmaniasis is a neglected disease caused by protozoa of the genus *Leishmania*. One of its characteristics is the imbalance of the host's immune response to favor the survival of the parasite, generating inflammatory processes that do not promote parasite control and induce tissue injury. In this context, the use of mesenchymal stromal cells (MSCs) and some of their biological products such as their Extracellular Vesicles (MSC-EVs) may be a viable therapeutic strategy, given the already described immunomodulatory potential they present. Our group demonstrated that treatment with MSCs from adipose tissue contributes to lesion control in C57BL/6 mice infected with *Leishmania amazonensis.* As the paracrine effect is one of the main mechanisms of efficacy of treatments with MSCs, this project aims to investigate whether extracellular vesicles these cells secrete have the property of controlling the lesion similar to full cell treatment. With this, the project aims to understand the effects of treatment with MSC-EVs from adipose tissue of C57BL/6 mice in the model of experimental leishmaniasis induced by *L. amazonensis*. The first step it's the purification and expansion of MSC cells from adipose tissue and extraction and characterization of it's extracellular vesicles. Next, we evaluate the *in vitro* effect of extracellular vesicles. Infected C57BL/6 macrophages were treated with MSC-EVs and the parasite load of these macrophages were evaluated. Finally, we evaluate the ability of MSC-EVs to control injury *in vivo* in infected C57BL/6 animals. The size of the lesions was evaluated by pachymetry, the parasite load by limiting dilution technique and the immune response by RT-PCR and ELISA. As preliminary results, MSC-EVs reduced lesion size without affect parasite load. MSC-EVs could be an alternative therapy for the treatment of leishmaniasis lesion. **Supported by:**CAPES, 88887.695120/2022-00
Keywords: Cellular Therapy;Extracellular Vesicles;Alternative therapy.

HP-56 - **Investigation of the role of the endosymbiont Wolbachia pipientis in the infection of the vector Lutzomyia longipalpis by the parasite Leishmania infantum chagasi**

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The transmission of American visceral leishmaniasis (AVL), caused by *Leishmania infantum chagasi*, occurs through the bite of the female sand fly *Lutzomyia longipalpis*.Current methods for leishmaniases control, as the use of insecticide, have been shown to be ineffective.This indicates the need to develop new strategies. The bacterium *Wolbachia pipientis* has been used to control arboviruses.Other studies point to a decrease in the infectivity of the protozoan *Plasmodium*, which causes malaria, in *Anopheles* mosquito in the presence of Wolbachia.A recent study of our group tested the infection susceptibily of *L. longipalpis* Lulo and LL-5 embryonic cell lines to the Wolbachia wMel and wMelPop-CLA strains.The study showed that the cells, when exposed to the bacterium, modulate genes belonging the main immunological pathways Toll, IMD and Jak-Stat.To investigate the role of Wolbachia in adult insects we now performed artificial blood feeding of the insects with Lulo cells stably infected with wMelPop-CLA.We then evaluated the permanence time of the bacterium in the vector *Lutzomyia longipalpis*, as well as the possible effects of a coinfection with *L. i. chagasi.*Preliminary results showed that it was possible to detect Wolbachia in the insect at 0h, 24h, 48h, 72h and 144h post-infection times, which proves the oral route to be a promising way of introducing the endosymbiont into the sand fly.We also evaluated the *L. i. chagasi* load in the presence and absence of Wolbachia, and we observed no significant difference in relation to the control infected only with *Leishmania*.In addition, the modulation of some targets for insect immunity genes is being evaluated, and in our first tests, we observed that in the group containing the bacteria there is a slight tendency for reduction of the expression of some of these genes in relation to the group without Wolbachia. These experiments are being repeated in order to obtain a reliable number of replicates and proper statistical data. **Keywords:**Vector control;Lutzomyia longipalpis;Wolbachia pipientis.

HP-57 - **Oral immunization using live bacteria expressing the LACK protein from Leishmania amazonensis.**

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Leishmaniasis are neglected diseases caused by protozoans of the Leishmania genus, divided into Cutaneous – most common, Mucosal and Visceral forms. To date, no injectable vaccine aimed at boosting protective Th1 responses has shown to be sufficiently effective and safe for human use. An alternative strategy is a tolerogenic vaccine using predominantly Th2 antigens to prevent the swift expansion of Th2 responses following infection, to allow the manifestation of protective Th1 responses. 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. An important Th2 component of LaAg is the LACK protein (Leishmania homologue of Activated C Kinase), highly conserved among Leishmania spp. In this work we produced and evaluated the potential use of non-pathogenic live bacteria expressing the LACK protein as an oral vaccine against Cutaneous Leishmaniasis (CL). To this end, the lack gene was successfully cloned into *Escherichia coli* DH5α bacteria, as shown by PCR, SDS-PAGE and Sanger sequencing. Next, BALB/c mice pre-treated or not with a cocktail of antibiotics received two oral doses with an interval of seven days with *E coli* expressing plasmid with the *lack* gene (pLACK+) or plasmid without *lack* (pLACK-). One week after the second vaccine dose, the animals were infected s.c. with *L. amazonensis* in the paw, and the lesion growth monitored periodically. We observed that animals pre-vaccinated with *E. coli* pLACK+ developed smaller lesions than those that received *E. coli* pLACK-. The pre-reduction of the intestinal microbiota with antibiotic therapy enhaced the vaccine effect of *E. coli* pLACK+ as confirmed by the parasite load measurement. Overall, these results show the viability of a live oral vaccine expressing the LACK protein gene against CL.
Supported by:CAPES **Keywords:**cutaneous leishmaniasis;live vaccine;LACK.

HP-59 - **Evaluation of ATP-citrate lyase expression in macrophages during *Leishmania amazonensis* infection**

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Leishmania spp. is an obligatory intracellular parasite that causes leishmaniases, and is transmitted to vertebrate hosts by sandflies of the genus *Phlebotomus* and *Lutzomyia*. Even though it is estimated that about 1 billion people worldwide are at risk of acquiring leishmaniases, it is still a neglected disease, and to this date, there is no available vaccine against leishmaniasis. Although there are available treatment options, they have problems such as adverse effects and high toxicity. Plus, there has been a rise in treatment failure and drug resistance reports. Thus, it is essential to investigate the parasite-host interaction to improve the treatment. Once inside the macrophages, parasites evade the microbicidal mechanisms and continue replicating and infecting other cells. They also induce alterations in host metabolism to favor their survival. It has been recently described that *Leishmania spp.* induce metabolic reprogramming and generate lipid droplets (LDs) in infected macrophages. Based on these findings, we investigate how the parasite modulates the host lipid metabolism. We hypothesized that during the infection, *L. amazonensis* induces an increase in lipids in macrophages and that this is due to the enzyme ATP-citrate lyase (ACLY) modulation. ACLY cleaves citrate in oxaloacetate and acetyl-CoA, and these two products can be used to produce inflammatory mediators like PGE2, NO, and ROS and cause epigenetic alterations like acetylation and malonylation. Our results show that in *L. amazonensis*-infected macrophages, there is an increase in lipids detected by Nile Red. When macrophages were pre-treated with the ACLY inhibitor BMS-303141, there was a decrease in ROS production in the early stages of the infection, but after 48 h, no effect was observed. ACLY inhibition modulated NO production and diminished 41% of parasite survival. These results indicate that the enzyme may be involved in the modulation of macrophage response to *L. amazonensis*. **Supported by:**CNPq **Keywords:**Leishmania amazonensis;ATP-citrate Lyase;macrophages.

HP-60 - **Leishmaniasis, Chagas disease and their vectors: representations and risk factors among Kariri-Xocó indigenous children**

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Trypanosomatid-transmitted protozooses affect populations with limited access to education and sanitation, such as indigenous. Children within these communities exhibit high capillarity and crucial role for prevention that necessitate a comprehensive understanding of local knowledge and reduce environmental risks. Thus, we investigated representations of Kariri-Xocó children (Bahia State) about leishmaniasis, Chagas disease, vectors, and the local risk factors. Ethical approval was obtained, and semi-structured interviews with the visual stimulus (*Lutzomyia* sp. and *Triatoma infestans* specimens) were conducted. Additionally, non-participant observation was used. Out of 21 interviewed children, 52% associated the sandfly with *Aedes* sp., and 62% were unaware of its habitat, eating habits, and preventive measures. Only 9% mentioned practices like eliminating standing water and tire care. Misconceptions were observed, with 4% relating the transmitted disease to zika and 9% to yellow fever. Symptoms reported included "balls on the body", fever (9% each) and headache (4%). Regarding Chagas disease, 33% recognized the insect as a kissing bug, 9% as a cockroach, and 57% lacked knowledge about its appearance and its habit. A minority (38%) mentioned hematophagy and said that the bug can be found in trees (19%), stones, walls (9% each), mud houses and sheets (4% each). The children's understanding of the disease transmission (95%) and its symptoms (62%) was limited, with only 4% citing fever, swollen heart, and death as consequences. Environmental risk included direct contact with forests and rivers, livestock farming, the presence of dogs, and improper waste management. The findings highlight significant knowledge gaps, misconceptions about disease severity and vectorial transmission, and the environmental risk within the community. Therefore, it´s require health education campaigns and community empowerment to mitigate risks and effectively preventing infectious diseases. **Supported by:**FAPESB
Keywords: Indigenous health;Environmental risks;Health education.

HP-61 - **Evaluation of nitroreductase type I bioactivation and antiamastigote activity of two nitroderivative chalcone-thiosemicarbazone hybrids in *Leishmania infantum***

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Recently, our group evaluated the antipromastigote activity against *L. infantum* and the cytotoxicity against murine peritoneal macrophages of 13 chalcone-thiosemicarbazone hybrids, obtaining IC_{50} values between 3.2 ± 0.9 and 23.3 ± 3.6 μM, and CC_{50} between 38.6 ± 1.8 and 99.3 ± 4.9 μM, except CT-7 and CT-14, which presented, respectively, CC_{50} equal to >512 and 143.9±12.6 μM. Interestingly, among the compounds tested, these were the only ones to present nitro groups in their structure. This led us to consider bioactivation through the nitroreductase type I (NTR1) enzyme as a possible mechanism of bioactivation. Thus, this work aimed to evaluate the bioactivation mechanism through NTR1 overexpression in *L. infantum*, and the antiamastigote activity of CT-7 and CT-14. The mechanism of bioactivation was evaluated through the antipromastigote activity in *Linf*WT (WT), *Linf*pSPaNEOa (PSP) and *Linf*pSPaNEOaNTR1^{high} (NTR1^{high}) strains, using nifurtimox as a control, and the antiamastigote activity was evaluated as a function of the reduction of the infection rate, in a concentration of 10 μM, concerning the infected control. As expected, nifurtimox showed a lower IC_{50} for the NTR1^{high} strain than for the WT and PSP strains (1.1±0.6, 2.0 ± 0.8, and 2.1 ± 0.4 μM, respectively). In contrast, CT-7 and CT-14 showed less significant IC_{50} reductions for the NTR1^{high} strain, when compared to the WT and PSP strains (CT-7: WT = 10.7 ± 2.5, PSP = 10.6 ± 1.2 and NTR1^{high} = 10.2±1.6 μM) (CT-14: WT = 9.0 ± 0.9, PSP = 8.7 ± 0.7 and NTR1^{high} = 6.6 ± 1.5 μM). In the antiamastigote activity experiments, reductions in the infection rates for CT-7 and CT-14 equal 88.2% and 87.7%, respectively, were observed. These results suggest that NTR1 does not bioactivate CT-7 and CT-14. As a continuation of this study, investigations will be carried out about the mechanism of action, in addition to the determination of IC_{50} and selectivity index of CT-7 and CT-14 against the amastigote form of *L. infantum*. **Supported by:**FAPERJ PROCESSOS E26-201.158/2022 E 26-210.157/2018 **Keywords:**Visceral Leishmaniasis;Leishmania infantum;Nitroreductases.

HP-62 -**Building the Neospora caninum GRAome: Bioinformatic and Proteomic strategies for the assembly of a Dense Granule Proteins database**

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Apicomplexa parasites require a parasitophorous vacuole (PV) for replication and infection maintenance. The PV is formed through protein secretion from organelles such as micronemes, rhoptries, and dense granules. Dense granule proteins (GRAs) maintain the PV structure and regulate host gene expression. Research has focused on GRAs of *Toxoplasma gondii*, but little is known about related *Neospora caninum*, which causes reproductive disorders in cattle and has a high impact on the livestock industry. In this study, we aimed to compare the known GRAs of *T. gondii* described in the literature and at the Toxoplasma Informatics Resources Database (ToxoDB), to *N. caninum* orthologues, in order to describe and annotate those genes and look for conserved or unique motifs. We initially found a total of 186 GRAs in *T. gondii* and, of these, 145 present orthologs in *N. caninum*, with a high level of synteny. The similarity between sequences was also analyzed through alignments and determined to be in the range of 40 to 80%. Gene expression of the GRAs were also analyzed in RNASeq experiments involving infections by these parasites in murine bone marrow-derived macrophages, where, a similar profile was observed in the gene expression of the GRAs in both parasites, with the exception of some genes. To validate the *in silico* strategy, we used proximity biotinylation to identify predicted targets in the *N. caninum* PV. As baits, we chose a known GRA with predicted good expression in tachyzoites (GRA48), and a knocked in *T. gondii* GRA without orthologue in *N. caninum* (GRA24). The BioID assays confirmed at least 57 predicted GRAs in *N. caninum*'s PV, including GRAs only predicted by HyperLOPIT and yet to be described in *T. gondii*. In conclusion, the union of bioinformatics and proteomics strategies is a successful method to describe the GRA content in the *N. caninum* infected cell, serving as a starting point for new experimental approaches regarding parasite biology and host interaction. **Supported by:**CNPq (313761/2020-5), FAPEMIG (RED-0313-16) **Keywords:**Toxoplasma gondii;RNASeq;BioID.

HP-63 - **Identification of aneuploidy and copy number variation (CNV) events in the TcCh39 chromosome of *Trypanosoma cruzi* (clone CL Brener) by dual color FISH assay**

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Aneuploidy appears to play a role in the protozoan parasite's adaptation to environmental changes, including drug resistance. Although *Trypanosoma cruzi* is considered a diploid organism, whole-genome sequencing and comparative genomic hybridization analyses have shown the occurrence of aneuploidy in this parasite. Here, we investigated the presence of aneuploidy in *T. cruzi* using dual-color fluorescence in situ hybridization (FISH), which allowed us to estimate the copy number of a given chromosome (somy) in individual cells. The single-copy genes H49 and JL8 were used as specific markers of chromosome TcCh39 of clone CL Brener. The central region of genes H49 and JL8 comprises an uninterrupted large array of tandem repeats that is a good DNA hybridization target. Hybridization signals for H49 and JL8 were revealed with anti-Digoxigenin and Alexa Fluor 488 and anti-Biotin and Alexa Fluor 594, respectively. Ploidy levels were also confirmed by confocal microscopy. Cells in mitosis were excluded from the analysis. We looked at changes in the chromosome TcChr39, specifically for chromosomal and local copy number variations. We identified the copy number chromosomal variation of TcCh39 in the same cell population: 55% of cells were disomic; 25.2%, monosomic; and 5.4%, trisomic for chromosome TcCh39. Trisomy or monosomy of TcChr39 could result from an error in chromosomal segregation in mitosis due to the non-disjunction of sister chromatids. Among disomic cells, some displayed local gene copy number variation. For instance, they had two copies of the H49 gene and just one copy of the JL8 gene. The lag in the replication of gene JL8 could explain this difference. The variable TcChr39 somy and copy number of genes among individual cells may have generated intra-strain heterogeneity and chromosomal mosaicism. We will compare the chromosome copy number changes estimated by FISH with data provided by next-generation sequencing of clone CL Brener. **Supported by:**Acknowledgement: FAPESP (PD 2019/05049-4) and Thematic Project (2016/15000-4), CAPES and CNPq. Elizabeth Naomi for help in handling the confocal microscopy (INFAR/UNIFESP).
Keywords: Trypanosoma cruzi;aneuploidy;copy number variation.

HP-64 - **A genetically modified live attenuated *Leishmania major* as a potential vaccinal candidate for Leishmaniasis**

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Leishmaniasis is a neglected tropical disease caused by more than 20 different species of parasites belonging to the genus *Leishmania*. The disease constitutes a global health problem, affecting approximately 1 million people annually worldwide. Brazil is an endemic country for leishmaniasis. Although treatment exists, it is generally highly toxic and costly. Furthermore, currently there are no vaccines approved for humans against this pathogen. In this study, we investigated the physiological characteristics of a genetically modified strain of *Leishmania major* in which a specific gene has been knocked out, and explored its potential application as a vaccine candidate. In culture, the KO promastigotes exhibited enhanced growth, reaching approximately $3.0x10^7$ parasites/mL compared to $2.5x10^7$ parasites/mL observed in the wild-type strain after 5 days of growth. Additionally, a lower percentage of metacyclic forms was observed in the KO strain (around 10%) compared to the wild-type strain (35%). Conversely, overexpression of this gene resulted in the opposite effect, with a higher percentage of metacyclic forms and reduced growth. Furthermore, the KO strain demonstrated attenuation during *in vitro* infection, exhibiting a lower percentage of amastigotes per macrophage. *In vivo*, subcutaneous infection with $2x10^6$ parasites in C57BL/6 mice did not result in any lesions, unlike infection with the wild-type strain. Additionally, Balb/C and C57BL/6 IFN-y-/- mice infected with $2x10^5$ parasites did not develop lesions, while those infected with $2x10^6$ parasites showed significantly smaller lesions compared to the wild-type strain. However, in both infections, it was possible to recover the KO strain from infected tissues. These data collectively demonstrate the high potential of this strain as a vaccine candidate. Currently, ongoing research is being conducted to evaluate whether these parasites can provide protection against infection by the wild-type strain. **Supported by:**FAPERJ / CNPQ **Keywords:**Leishmania;Leishmanization;Vaccine.

HP-65 - **Analysis in vitro of RNA as a molecular marker of *Trypanosoma cruzi* viability**

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Real-time polymerase chain reaction (qPCR) allows the detection and quantification of different pathogens and are widely employed in molecular diagnostic laboratories. However, DNA amplification does not differentiate between viable or dead parasites. Until now there are few studies comparing the application of molecular diagnostic tools that effectively differentiate between viable and nonviable parasites especially when it comes to *T. cruzi* evaluation. Herein, we considered *T. cruzi* RNA as a potential molecular marker of pathogen viability, due to RNA's half-life and lability when compared to the DNA. At first, we compared a qPCR with Reverse Transcription (RT-qPCR), targeting *T. cruzi* GAPDH mRNA (a housekeeping gene), and a qPCR, targeting *T. cruzi* satellite DNA. Both methodologies presented an improved performance with linearities ranging from 10^7 to 10^2 parasites equivalents for RT-qPCR and 10^6 a 10^{-1} parasites equivalents for qPCR and efficiencies of 100.3% and 102.6%, respectively. Then, we evaluated and compared RNA and DNA detection of live *T cruzi* and heat-treated lysed *T. cruzi,* where was confirmed that RNA was faster degraded, no longer being detected at day 1 after parasite lysis, while DNA detection was stable with no decrease in parasite load over the days even after parasite lysis. Moreover, *in vitro* assays with rat cardiomyoblasts (H9C2) infected with *T. cruzi* (Dm28c) and under Benznidazole treatment, also showed a considerable decrease in RNA detection, which was no longer detectable at day 1 post-treatment. At the same time, parasite DNA detection remained constant with no significant decrease in its detection up to 4 days after treatment. In conclusion, these differences between DNA and RNA detection raises the possibility that RNA is a potential molecular marker of *T. cruzi* viability, which could contribute to understanding the dynamics of the parasite infection and explore new possibilities for trypanocidal drugs evaluation. **Supported by:**IOC-FIOCRUZ; CnPq; FAPERJ
Keywords: Trypanosoma cruzi;Viability;qPCR, RT-qPCR.

HP-66 - **Leishmania antigen 2 (Leish-Ag2) induced protection by intranasal route against *Leishmania amazonensis* infection**

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Leishmaniasis is a neglected tropical disease caused by the infectious protozoa *Leishmania*. The various species of Leishmania cause cutaneous (CL) and visceral (VL) leishmaniasis. According to the Pan American Health Organization (PAHO), over 12 million people worldwide are infected, resulting in 20,000 to 30,000 deaths annually. Developing a vaccine for effective disease control is the most viable solution to address this health issue. However, there is currently no approved vaccine against leishmaniasis for human use. In the literature, several potential candidates have been studied to advance research in this field. In this study, we evaluated the efficacy of *Leishmania* antigen 2 (Leish-Ag2) administered via the intranasal route in a BALB/c mice model of experimental *Leishmania amazonensis* infection. Leish-Ag2 is an antigen that is well-preserved across different species of Leishmania and has recently been studied by our research team, with its findings being kept confidential. By itself, Leish-Ag2 was unable to induce protection in the tested model. However, when the antigen was combined with adjuvants such as MPLA, Leish-Ag2 demonstrated a significant reduction in lesion size and parasite load. This protective effect is likely linked to the adjuvants' ability to modulate a Th1 immune response in BALB/c mice, thereby enabling control of the infection. This is supported by data from our research group, indicating high levels of IFN-γ at the site of the lesion in animals vaccinated with Leish-Ag2 associated to MPLA. Taken together, our research group has developed a new antigen with the capacity to protect against cutaneous infection when administered via the intranasal route. Furthermore, there is potential for the mucosal route to induce protection against such an infection. **Supported by:**CNPq **Keywords:**Leishmaniasis;Vaccine;Intranasal.

HP-67 - **A novel fatty acid binding protein in *Leishmania amazonensis*: characterization of the LeiFABP domain**

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Leishmaniases are diseases caused by parasites of the genus *Leishmania*, of the Trypanosomatidae family. During their life cycle, *Leishmania* parasites multiply in vertebrate and invertebrate hosts, differentiating into distinct cell forms: promastigotes and amastigotes. These parasites have limited biosynthesis capacity, having mechanisms for sequestring nutrients from the host. The amastigote forms depend on fatty acids and lipids present inside the phagosome, which are the main sources of carbon used for production of molecules essential for their survival. Searches on TriTrypDB for hypothetical proteins similar to eukaryotic fatty acids binding proteins (FABP) led to the hypothetical protein encoded by *LmxM.34.3070*. Different protein structure prediction methods revealed a C-terminal domain structurally similar to FABPs, suggesting that LmxM.34.3070 could be associated with the metabolism of lipids and fatty acids. Also, BLAST alignments showed that the *LmxM.34.3070* is strongly conserved among the *Leishmania* genus. Therefore, the main goal of this work was to obtain the recombinant C-terminal domain of LmxM.34.3070 suitable for structural biology studies. The fragment LmxM.34.3070$_{638\text{-}822}$ (LeiFABP), was cloned, expressed, and purified. Circular dichroism spectroscopy data indicated that 32% of LeiFABP amino acids assume a β-type structure, while 15% assume an α-helix structure. The result of this analysis is consistent with the early *in silico* predictions. Multiangle light scattering showed that the LeiFABP domain is monomeric. The ^{1}H Nuclear magnetic resonance (NMR) spectrum showed good dispersion in the HNs region and relatively narrow lines, suggesting that the LeiFABP construct is well folded and is suitable for structural analysis by NMR in solution or crystallography. Supported by CAPES and Fapesp.
Supported by:CAPES PROEX , PROCESSO: 88887.613399/2021-00
Keywords:fatty acid binding protein;circular dichroism spectroscopy;nuclear magnetic resonance.

HP-68 - **Repositioning oral treatment for visceral leishmaniasis managed by molecular docking**

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Molecular docking is a great tool for the drug repositioning strategy. Considering visceral leishmaniasis is a disease that leads to death in around 90% of non- treated patients, the quick development of an oral effective treatment would save thousands of lives. Some macromolecules that are key to Leishmania spp. survival and pathogen-specific have been described as good treatment targets. This work presents a target-directed virtual trial, to find repositionable oral drugs that are potent inhibitors for an enzyme involved in the viability of the parasite. Due to the possibility of the deposit of patents, sensitive data related to the invention was omitted. A library of around 4300 compounds was screened and ranked by the binding affinity with the specific L. infantum enzyme. The 3D structures are available in the ZINC database. The Raccoon software was used to input ligands charges. For the receptors, two physiological forms of the enzyme were obtained by RCBS-PDB. The protonation state in 7.4 pH was normalized at the APBS server. The molecular docking was calculated with PyRx software. Both states of the enzyme were tested by all the compounds binding in their active site, creating two rankings on the affinity. The first enzyme form had 1118 compounds in conformations with binding values equal to or under -8 Kcal/mol. The second form had 504 molecules at the same range, of which, 475 had conformations in this binding affinity with both forms. These compounds were analyzed by their ADMET properties, where 239 orally administered drugs were found, of which 54 were approved by the FDA and ANVISA. The molecular mechanism of action shows that many of those interactions occur with important residues in the catalytic site of the enzyme. Taken together, our data proposed that those drugs may be a key to improve the treatment of leishmaniasis by competitive inhibition to a relevant macromolecule for the parasite's metabolism as a part of the mechanism of action. **Supported by:**CAPES, CNPQ, IOC/Fiocruz, FAPERJ
Keywords: Structure-based virtual screening;visceral leishmaniasis;chemotherapy.

HP-69 - **LAMP-CAS12 METHODOLOGY FOR MOLECULAR DIAGNOSIS OF INFECTIOUS DISEASES**

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Infectious diseases are a global health problem that impact billions of people, causing an immense burden of disabilities and deaths, affecting the economic and social dynamics of all the continents. The detection of its etiologic agents is necessary both for treatment of the patients in a timely manner and prevention of spread of the pathogens. Many approaches based on nucleic acid detection have been used to enhance the sensibility and specificity of the tests, reducing limitations such as the use of high-cost equipment and the delay in delivering results. Among the strategies recently tested and approved by the U.S Food and Drug Administration (FDA), is the use of the LAMP (Loop-Mediated Isothermal Amplification) methodology, alone or combined with CRISPR/Cas12 technology. We established the LAMP-Cas12 methodology using as a model the detection of SARS-CoV-2. In addition, we developed in our laboratory all the steps for expression and purification of Cas12a enzymes, as well as the synthesis of their sgRNAs, comparing their efficiency in the detection of SARS-CoV-2 in in vitro assays. We also validated the assays that use the nonspecific activity of Cas12a after detection of the target of interest, coupled to a fluorescent molecule to confirm the diagnosis. Finally, we performed a proof of concept of this methodology, using samples from patients diagnosed with COVID-19 previously confirmed by qRT-PCR, and demonstrated that the system is able to detect with specificity different types of target. Now, we will use these established diagnostic platform to establish new protocols for the detection of other infectious disease agents, including protozoans, worms and bacteria. **Keywords:**Infectous disease;CRISPR;Diagnostic.

HP-70 - **UNRAVELING THE FUNCTION OF GLYCOCONJUGATES IN LEISHMANIA AMAZONENSIS INFECTION THROUGH CRISPR/CAS9 EDITING OF THE LPG2 GENE**

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Introduction: The *lpg2* gene encodes a GDP-mannose transporter involved in synthesizing phosphoglycan-containing molecules (PGs), which are critical for *Leishmania* infection of the mammalian host. This study investigated the role of these glycoconjugates in 2 strains of *L. amazonensis* (*La*) previously isolated from patients with localized (LCL BA125) or diffuse (DCL BA336) forms of the disease. **Methods:** *La lpg2*KO strains were generated using CRISPR/CAS9 and clonal *lpg2*KO parasites were isolated. Neutrophils and BMDM were infected with WT or *lpg2*KO parasites, and parasite burden was determined at 4h or 48h post-infection. Cellular migration assays were performed with human dendritic cells (hDCs) infected with WT or *lpg2*KO parasites *In vivo.* Evaluation involved the intradermal inoculation of parasites in the ear of BALB/c mice, and lesion development was monitored using an analog caliper for up to 12 weeks. **Results:** The disruption of the *lpg2* gene was characterized by PCR, Sanger and next generation sequencing. Western blot analysis demonstrated the complete absence of LPG and PG expression. In vitro assays showed no differences in infection and replication rates between *lpg2*KO and WT parasites in BMDM or human neutrophils. However, both *La lpg2*KO strains exhibited reduced hDC migration. Surprisingly, in vivo infection of mice yielded contrasting outcomes between the two lpg2KO strains. The BA336 *lpg2*KO strain displayed minimal differences in lesion development, while the BA125 *lpg2*KO strain failed to induce any lesion development. **Conclusions:** Our findings demonstrate successful disruption of the *lpg2* gene in both strains of *La*. The absence of PGs does not appear to be essential for parasite virulence in vitro, although it impacts hDC migration. In contrast, PGs seem to play an essential role in lesion development of BA125. Further research is needed to understand the factors responsible for the divergent in vivo virulence observed between these strains. **Supported by:**FAPESB, CAPES
Keywords: Leishmania;CRISPR/CAS9;LPG.

HP-71 - **Targeting different portions of trans-sialidase for the development of Chagas disease vaccine**

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Trans-sialidases (TS) are proteins present on the surface of *Trypanosoma cruzi*, which only a subgroup of TS has catalytic activity, responsible for transferring sialic acid residues from host glycoconjugates to mucins on the parasite surface, a mechanism that is related to the parasite capacity to evade the host immune system. Some TS have a C-terminal domain containing 12 amino acids repeats known as SAPA (shed acute parasite antigen). In addition, the SAPA domain increases the stability of the enzyme in the bloodstream, which is considered a parasite virulence factor. To evaluated an active TS as vaccine candidate, three recombinant versions of the protein were produced in *E. coli*: full-length protein, TS without repeats, and only SAPA repeats. BALB/c mice were immunized with each protein and then challenged with a virulent strain of *T. cruzi*. Analyzes of the cellular immune response showed that immunization with TS without SAPA resulted in higher levels of IFN-γ and lower levels of IL-10 produced by splenocytes from animals, compared to splenocytes from animals immunized with the other two antigens. Furthermore, after challenge, mice immunized with protein containing only SAPA repeats resulted in higher parasitemia and mortality compared to immunization with TS without SAPA. It is important to emphasize that tissues of animals immunized with TS without SAPA did not show inflammatory infiltrate or detectable levels of parasite DNA in the heart. Taken together these results indicated that immunization with TS antigen without SAPA induces the development of a protective Th1 response, essential for intracellular pathogen infection control, and that the presence of SAPA repeats results in the negative modulation of this protective response. Since RNA vaccines have several advantages and TS without SAPA is a promising antigen for a vaccine model against Chagas disease, the mRNA of this protein was produced and tested in a lipid formulation for animal immunization. **Supported by:**CAPES **Keywords:**Trans-sialidase (TS);SAPA repeats; T; cruzi.

HP-72 - **THE ROLE OF ANNEXIN A1 IN AN EXPERIMENTAL *TRYPANOSOMA CRUZI* INFECTION**

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Chagas disease (CD) is a neglected disease caused by the protozoan *Trypanosoma cruzi (Tc)*, whose complications include cardiac, digestive and neurological dysfunction. The imbalanced inflammatory response is associated with the clinical forms development, thus Annexin A1 (ANXA1) emerges as an important protein with a central role in the inflammatory process resolution. Herein, the influence of ANXA1 on gut microbiota composition and in the development of Tc-induced pathogenesis were investigated. For *in vivo* analysis, female BALB/c (WT) and ANXA1 knockout (KO) mice, 8 to 9 weeks old, were infected ip with 10^3 trypomastigotes forms (Y strain). Parasitemia, body weight and survival were evaluated. We collected fresh stool samples at 10 and 20 day post-infection (dpi) for cultivable fecal microbiota analysis. For *in vitro* analysis, we used the primary myenteric neurons culture assays. The supernatant was collected at 24, 48 and 72 hpi with *Tc* Y strain (10:1) for lactate dehydrogenase (LDH), nitric oxide (NO) and ELISA for the cytokines IL-6, TNF-α, IFN-γ and IL-10. The results showed that the deficiency of ANXA1 resulted in higher parasitemia, weight loss, and mortality rate compared with WT. The cultivable fecal microbiota quantification showed that ANXA1 KO mice had less *Bacteroides*, *Staphylococcus* and *Enterococcus* at 10 and 20 dpi. *Staphylococcus* in WT mice decreased with *Tc* infection, whereas it increased in ANXA1 KO mice. *In vitro*, at 24 hpi, myenteric neurons from infected-ANXA1 KO mice showed higher levels of NO, IL-6, TNF-α and IFN-γ and lower levels of IL -10 production compared with WT. Collectively, these results suggest that ANXA1 is a vital regulator of the development of CD pathogenesis, which may be influenced by the intestinal microbiota affecting communication between gut/immune/central system. This helps portraying new insights into disease pathogenesis and may provide new potential therapeutic approaches. **Supported by:**CNPq, FAPEMIG e CAPES
Keywords: Trypanosoma cruzi;Annexin A1;Microbiota.

PV-13 - **The expression of positive and negative mutants of TcRlp, a Ras-like GTPase of *Trypanosoma cruzi,* suggests a role for this protein in epimastigote proliferation and metacyclogenesis**

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Ras family GTPases act as molecular regulators of essential cellular processes, such as cell proliferation and differentiation, alternating between active (GTP-bound) and inactive (GDP-bound) stages. These GTPases are also modified by isoprenylation (geranylgeranylation or farnesylation) in a CaaX motif that allows association with membranes. The parasite *Trypanosoma cruzi* has only one Ras family gene, which encodes the protein TcRlp and has a CaaX CVLL motif, which is geranylgeranylated. Previously, the TcRlp gene was cloned from the *T. cruzi* (Dm28c) and several mutant versions were produced, including: TcRlp-Q61K (positive dominant), TcRlp-S17N (negative dominant) and TcRlp-CQLF (farnesylated mutant). The wild-type TcRlp gene and its mutants were cloned into the pTEX-GFP plasmid, allowing for their expression with a GFP tag. This work investigates the impact of TcRlp mutants in *T. cruzi* biology, assessing cell localization of mutant proteins by fluorescence microscopy, evaluating the epimastigote proliferation potential through growth curves on LIT and assessing metacyclogenesis capacity with the TAU-3AAG assay. The microscopy analysis reveals that the TcRlp-S17N and -CQLF are mostly localized in the nucleus, while TcRlp-WT the -Q61K display predominant cytoplasmic localization. The growth curve revealed that cells expressing TcRlp-Q61K present a lower growth, when compared to GFP control, in the presence of either 1 or 10% FBS, while TcRlp-S17N displays higher proliferation, which is more apparent in 1% SFB. Analysis of trypomastigote/epimastigote proportions at 48 and 96 hours of TAU-3AAG stimuli revealed that metacyclogenesis increases in cells expressing either the TcRlp-S17N or -Q61K mutants, and that the proportion of metacyclics to epimastigotes for -Q61K is ~1.5 times higher than the observed for -S17N. The results obtained in this study points toward a role for TcRlp as a putative negative regulator of cell grown and positive promotor of metacyclogenesis.
Keywords: Ras family GTPases;Trypanosoma cruzi;Metacyclogenesis.

PV-14 - **Evidencing centromeric regions in Trypanosoma cruzi: an in silico and experimental approach**

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Trypanosomatids have a peculiar genome organization and transcription is arranged in polycistronic transcription units (PTU). Mitosis in T. cruzi occurs with chromosome semi-condensation, hampering classical cytogenetic approaches. Few studies explored the relationship between centromeric regions and kinetochore proteins governing chromosomal segregation. Centromeres have been studied through high throughput (Hi-C) analysis. Hi-C data reveals a genome-wide DNA-DNA contact matrix, enabling PCA analysis for centromeric region identification. Investigating T.cruzi Brazil A4 Hi-C data (Wang et al 2021), we highlighted 11 putative centromeric regions. Canonical eukaryotic kinetochore proteins have not been identified in trypanosomatids, but 25 kinetoplastid kinetochore proteins (KKT) were described in T.brucei (Akiyoshi B, Gull K. 2014). We focus on characterizing centromeres using PCA patterns and two KKTs constitutive of centromeric DNA. In silico investigation involves BLASTn searches against centromeric DNA of T. cruzi CL brener, synteny analysis, and genomic features (PTU directions and GC content). These strategies identify the whole centromeric region of T. cruzi CL Brener chromosome 3 on chromosome 20 of T. cruzi Brazil A4. We also found the typical PCA pattern from Hi-C analysis, representing DNA-DNA interactions at the chromosome arms. We will validate T. cruzi putative centromeric regions by generating labeled T. cruzi strains for two KKT proteins and determining their genomic location by Chip-seq. In summary, we expect to confirm Hi-C's ability to inform centromere location in T. cruzi through experimental analysis, enhancing our understanding of genome organization in trypanosomes. **Supported by:**FAPESP-Processo 2022/12897-4 **Keywords:**Centromere ;Kinetochore;Hi-C.

PV-15 - **Isolation of active ribosomes by RiboLace enables the study of *Leishmania* translational adaptation**

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Protozoan parasites of the genus *Leishmania* exhibit great phenotypic variability in terms of clinical symptoms, illness prognosis, and treatment susceptibility, complicating disease management. Such variety is impressive considering the parasites' constitutive gene expression, which lacks traditional promoter-driven regulation, raising questions on how *Leishmania* adapts and develops in response to environmental change. Using an experimental evolution approach, we have discovered various regulatory mechanisms that govern *Leishmania* fitness gain in culture, including gene dosage changes caused by genomic instability, and compensatory responses at post-transcriptional and translational levels. These findings point to the presence of fitness-adapted ribosomes, which may aid parasite adaptation by filtering out the effects of deleterious gene dosage effects while promoting the effects of beneficial ones. Here we use RiboLace (Immagina Biotechnology) to specifically profile only active ribosomes to investigate adaptive changes in mRNA translatability. We initially tested the method's quantitative application by creating *L. donovani* transgenic parasites that express different levels of GFP caused by modifications in the Kozak sequences (CTTTA: intermediate expression, LdGFP_I; CCACC: high expression, LdGFP_H) and the insertion of hairpins at the 5'UTR (low expression, LdGFP_L), both of which impact mRNA/ribosome interaction. Flow cytometry analysis showed a 4-fold reduced mean fluorescence intensity in LdGFP_I parasites compared to LdGFP_H. RiboLace-isolated, active ribosomes from both LdGFP_I and LdGFP_H strains showed a 2.74-fold higher recovery of GFP mRNA in the latter parasites, thus validating the applicability of RiboLace to quantify differences in translatability. We are currently employing this approach to investigate the role of translational control and the existence of fitness-adapted ribosomes in *Leishmania* during adaptation to *in vitro* culture. **Supported by:**This project is supported by a grant from the Institut Pasteur 'Programmes Transversaux de Recherche' (PTR 425-21) and European Research Council (ERC) Synergy grant for the project 'DECOLeishRN – Decoding epistatic genome/RNome interactions in eukaryotic fitness gain using Leishmania parasites as a unique model system', Grant agreement ID: 101071613
Keywords: Leishmania;adaptation;ribosome.

PV-16 - **The dose makes the poison: analyses of the possible cytotoxicity of thymidine analogs used to monitor DNA replication in trypanosomatids**

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Thymidine analogs (5-Bromo-2′-deoxyuridine – BrdU and 5-Ethynyl-2'-deoxyuridine – EdU) are widely used to monitor DNA replication in eukaryotic cells. However, recent articles have been reporting certain levels of cytotoxicity in mammalian cells, which can bias data related to the replication capacity of the analyzed cells. In trypanosomatids, there are no reports of toxic effects related to the use of thymidine analogs. Thus, there are three possibilities: BrdU and EdU are not toxic for these parasites at standard working concentrations; BrdU and EdU are toxic at standard working concentrations and no research group has evaluated this toxicicity; or only one of these analogs is toxic. To find out which of these possibilities is correct, we investigated the possible cytotoxic effects of BrdU and EdU on *Trypanosoma cruzi*, *Trypanosoma brucei*, and *Leishmania major*. Preliminary data suggest that EdU is toxic at working concentrations to *L. major*. Considering that the only difference between EdU and BrdU is the presence of the alkyne group in place of the bromine atom in position 5, this preliminary result justifies the beginning of a further investigation into the cytotoxicity of alkyne groups present in the DNA. Currently, we are treating the trypanosomatids with different concentrations of BrdU and EdU and evaluating their growth curves, concomitantly with cell viability assays. Moreover, we are investigating the presence of possible cell cycle arrests by the analysis of DNA content using flow cytometry. We also intend to investigate the presence of DNA damage and DNA damage response through IFA using α-γH2A, and by TUNEL assay. If we find DNA damage, we intend to track the regions of the genome where damage is occurring by ChIP-seq using α-γH2A. Of note, a better understanding of the essential molecular mechanisms during the life cycle of these organisms can contribute to the development of specific strategies to combat the diseases caused by these parasites. **Supported by:**Higher Education Improvement Coordination (CAPES), and São Paulo Research Foundation (FAPESP) **Keywords:**BrdU and EdU;DNA replication and damage;Trypanosomatids.

PV-17 - **THE ROLE OF TCCSB IN MITOCHONDRIAL DNA REPAIR ASSOCIATED WITH TRANSCRIPTION IN *TRYPANOSOMA CRUZI***

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Trypanosoma cruzi, the cause of Chagas disease, has unique mitochondria. Nucleotide excision repair pathway in this organelle remains uncharacterized. In this study, we investigated the role of CSB, a gene involved in transcription-associated nucleotide excision repair. Cells modified for the CSB gene (single knockout or overexpressing) were exposed to Doxorubicin (targets mitochondria - MtDOX, or nucleus - Dox) and cisplatin (both organelles). After exposure to of MtDox, TcCSB-deficient cells showed increased sensitivity 1h after treatment compared to the wild-type cells, but resumed their growth after 24h. On the other hand, TcCSB overexpressing cells exhibited increased resistance 1h after treatment compared to WT cells, and their growth was halted. After treatment with of Dox we observed that the TcCSB-overexpressing cells were more sensitive, and the TcCSB-deficient mutant was more resistant 1h after treatment, although the former resumed their growth more quickly. When treated with of cisplatin, the same response as with Dox was observed. We then analyzed the repair kinetics with cisplatin and found that the TcCSB-overexpressing cells repaired the mitochondrial damage more rapidly, while the TcCSB-deficient cells had slower repair. We also evaluated changes in mitochondrial transcription after cisplatin treatment and observed that TcCSB-overexpressing cells showed a decrease in transcripts immediately after treatment. After 1h of treatment, the overexpressing cells had already recovered the transcript levels, while the levels continued to decline in the other strains, with a more pronounced decrease in the TcCSB-deficient strains. These results suggest the involvement of TcCSB in mitochondrial repair, and its absence somehow impairs the metabolism of kDNA in *T. cruzi*. The data show that unlike all organisms studied so far, *T. cruzi* has a DNA repair pathway associated with transcription, which may be related to the fact that it has only one mitochondria. **Supported by:**CNPq, FAPEMIG e CAPES
Keywords: Trypanosoma cruzi;kDNA;Mitochondrial DNA repair.

PV-18 - **Iron Overload could Induce Ferroptosis in *Trypanosoma cruzi***

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More than a century after its discovery, Chagas Disease is now considered an emerging disease due to the growing number of cases in non-endemic countries. The etiologic agent *Trypanosoma cruzi* is the object of intense studies worldwide and essential cellular and molecular mechanisms for the parasite's life, as is the case of ionic transport processes. One of these processes is the transport of iron (Fe), considered a critical ionic species in all living organisms, especially those related to energy metabolism, ATP synthesis, DNA synthesis, and the production of reactive O_2 species (ROS). Due to its shallow redox potential, Fe is both necessary and harmful, "friend and foe," depending on the conditions of its use by the cells. Fe overload could induce lipid peroxidation, inducing an iron-dependent cell death called Ferroptosis. Recently, we described an ionic Fe transport mechanism for *T. cruzi* involving two primary proteins: a Fe-reductase essential for reducing Fe^{2+} of the Fe^{3+} found in the extracellular environment and an iron transporter, internalizing Fe^{2+} as substrate. In addition, we described that exogenous Fe is required for proper signaling to control parasite proliferation and H_2O_2 formation, which stimulate parasite differentiation, thus interfering with parasite virulence, comparing parasites submitted to Fe depletion. We aim to evaluate the effect of exogenous Fe overload on the proliferation of epimastigotes of *T. cruzi*, on the regulation of ROS production, and the functional state of antioxidant enzymes, focusing on the possible involvement of the parasite's mitochondria in these processes and the role of Fe in ATP synthesis. Fe overload leads to the arrest of epimastigotes proliferation, and this effect is reverted using Ferrostatin-1, a potent inhibitor of ferroptosis. These results might indicate that Fe overload induces cell death in *T. cruzi*, probably via ferroptosis. **Keywords:**Ferroptosis;parasite lipid content;parasite oxidative stress.

PV-19 - **Investigation of the potential SUMOylation of RPA and its involvement in the nuclear export of this protein in infective life stages of *Trypanosoma cruzi***

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Chagas disease is a prevalent health issue in Latin America and it is crucial to understand the biology of its etiological agent, *Trypanosoma cruzi*. During its life cycle, the parasite alternates between replicative and infective forms and a key component in this context is Replication Protein A (RPA), which plays a vital role in genomic stability and DNA replication. *T. cruzi* relies on three RPA subunits and our previous work highlighted that RPA-1 and RPA-2 are exported from the nucleus to the cytoplasm in its infective life stages, and blocking this export hampers the differentiation. However, the molecular mechanisms underlying this export remain poorly understood. This study aims to shed light on them.
Through *in silico* assays, we have developed three-dimensional models of the RPA complex and found a nuclear export signal (NES) in the RPA-2 subunit, implying its potential involvement in RPA export during *T. cruzi* differentiation. Additionally, we have identified potential SUMOylation sites that, through molecular dynamics simulations, we have uncovered to stabilize the protein, leading us to speculate about its potential involvement in the exposure of the NES through conformational changes.
To validate these findings, we are employing a genetically modified bacterial strain capable of producing SUMOylated proteins. This system involves the introduction of plasmids containing the SUMO protein gene from *Trypanosoma brucei* (68% identity with *T. cruzi* SUMO protein) along with enzymes responsible for activating and ligating SUMO to the target protein. By introducing RPA-1, RPA-2, and RPA-3 genes into this system, which are already inserted in separated pET28a+ plasmids, we can co-express them. The potential SUMOylation of RPA will be examined through immunoblotting of lysed cells, where the presence of higher bands will indicate SUMOylation of the protein. **Supported by:**FAPESP - Processo 2021/12872-9 **Keywords:**Trypanosoma cruzi;Replication Protein A;SUMOylation.

PV-20 - **Endosymbiosis in trypanosomatids: the symbiotic bacterium regulates the oxidative metabolism of the host *Angomonas deanei***

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The coevolution of *Angomonas deanei* with a symbiotic bacterium represents an important model for studying the origin of organelles in the eukaryotic cell. In this mutualistic relationship intense metabolic exchanges occur between both partners and the symbiont completes essential biosynthetic pathways of the host protozoan. In this work, wild type (symbiont-harboring cells - AdWt) and aposymbiotic (AdApo) strains of *A. deanei* were compared under different nutritional conditions in order to investigate the influence of the symbiont on the intermediary and energy metabolism of *A. deanei* Data obtained by transmission electron microscopy show that AdWt presents marked ultrastructural alterations when compared to AdApo, especially in the mitochondrion and after cultivation in medium with proline. The O_2 consumption was 30% higher in the AdWt strain than in AdApo when cells were grown in the presence of glucose or proline. Both strains did not show significant variation in ATP levels after growth under such nutritional conditions. Data obtained by proteomic analysis indicate that the enzymes of the Krebs cycle, as well as those of the glycolytic and fermentation pathways are more expressed in AdApo and those of oxidative phosphorylation in AdWt. Taking together, data indicate that the symbiont promotes the recovery of intermediate metabolites, regulating and optimizing the oxidative metabolism of *A. deanei*. **Supported by:**88887.604982/2021-00 **Keywords:**endosymbiosis;intermediate metabolism;energy metabolism.

PV-21 - **Role of phosphorylation of Orc1Cdc6 protein in the cell cycle of *Trypanosoma cruzi***

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The process of DNA replication in the cell cycle requires strict control to ensure genomic stability. The pre-replication complex (PRC) performs this control, being formed, in most eukaryotes, by the Orc hexamer and the proteins Cdc6 and Cdt1, which act together to allow the action of the MCM complex. On the other hand, the replication process of *Trypanosoma cruzi* (*T. cruzi*), the etiologic agent of Chagas disease, is poorly understood. Its life cycle involves replicative (epimastigotes and amastigotes) and non-replicative forms (blood and metacyclic trypomastigotes) and has a protein, named Orc1Cdc6, composing the PRC. Orc1Cdc6 is present in the nuclear space in all stages of the life cycle, but without interacting with DNA in non-replicative forms, raising the hypothesis of possible post-translational modifications (PTMs) involved in the control of the interaction of Orc1Cdc6 with DNA. To investigate this hypothesis, we searched for possible phosphorylation sites in the Orc1Cdc6 protein (TriTrypDB Database code Tc508239.10) on the NetPhos website, resulting in 38 possible sites after data processing. We analyzed the same sequence on the PhosTryp website, which resulted in 8 possible phosphorylation sites in common. We also carried out structural modeling of the protein using the PyMOL software, which showed that 7 of the 8 sites found are exposed in the protein, being susceptible to phosphorylation. For in vitro analysis, parasites of the CL Brener strain with three myc tags was generated (TcOrc1/Cdc6_3myc) and submitted to immunoprecipitation and Phostag-gel assays, in epimastigote and metacyclic forms. Western blotting of the phostag gel revealed a differential migration of the Orc1Cdc6 protein from different morphologies. Thus, our data show that Orc1Cdc6 is differentially phosphorylated during the life cycle of T. cruzi. The biological implications of these modification(s) will be investigated. **Supported by:**Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Processos 20/00694-6 e 23/00557-7 **Keywords:**Trypanosoma cruzi;Orc1Cdc6;Phosphorylation.

PV-22 - **Unveiling Cellular Structures in Human and Veterinary Pathogens: A Closer Look with Expansion Microscopy**

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The Expansion Microscopy (ExM) technique offers a unique advantage by surpassing the limitations of traditional light microscopy, particularly the diffraction limit of light (~250 nm), leading to great advances in various fields of interest, one such area is the refinement of protein localization in pathogens. Is based in the use of a super hydrophilic polymer that permeates and make crosslinks with the biological sample, resulting in the formation of a swellable gel when in contact with water. The proteins of the sample become anchored to the polymer network due the crosslinks, allowing for an isotropic expansion of the specimen. Our initial focus was on analyzing the cytoskeleton of two human pathogens, *Trichomonas vaginalis* and *Trypanosoma cruzi*, by labeling tubulin in expanded cells. Additionally, we aimed to investigate the unique structure called costa, a prominent striated fiber found in *Tritrichomonas foetus*, a parasite that affects cattle and cats. As a result, we successfully achieved expanded cells with an expansion factor of approximately 4.6x. In all three types of cells, the labeled cytoskeletal protein complexes displayed stretching in all directions without any signs of fragmentation, maintaining the proportional integrity of the parasites. However, it is worth noting that membrane proteins did not exhibit successful labeling after expansion. This aspect necessitates further modifications to the protocol, such as considering an alternative denaturation process that does not compromise these membrane components, such as the plasma membrane. Additionally, this technique can be applied to whole cells and purified fractions for nanostructure research. The stability of the gels allows for their utilization up to 15 days later for additional labeling. Further studies using ExM can help understand the relationship of these protein components with other structures and organelles. **Supported by:**FAPERJ **Keywords:**Expansion microscopy;Cytoskeleton;Fluorescent imaging.

PV-23 - **COPPER CHLORIDE AND SULFATE SOLUTIONS AGAINST EPIMASTIGOTES OF *Trypanosoma cruzi* (CHAGAS, 1909): AN *IN VITRO* PRE-CLINICAL DATA**

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Chagas disease (CD) is caused by the protozoan *Trypanosoma cruzi* affecting 6-7 million people worldwide, mainly in Latin America. Treatment of CD is based on the use of benznidazole and nifurtimox. However, both drugs present low efficacy during the chronic phase of CD, causing severe side effects. Therefore, a high-efficacy less-toxic treatment for CD is urgently needed. Pre- and clinical trials have proven the “biocide” effect of copper, but rarely in *T. cruzi*. This study aimed to test copper sulfate ($CuSO_4$) and copper chloride ($CuCl_2$) against epimastigotes from the Dm28c clone of *T. cruzi*. The parasites (2e6 cells/mL) were incubated with the solutions (Cu-II) in different concentrations (0 – 2000 $\mu mol^{-1}/L$) for nine days at 28°C and counted daily in a Neubauer chamber. Parasites incubated with only LIT medium were considered negative control, and a group treated with paraformaldehyde 4% was considered positive control. The antiparasitic activity was evaluated by the daily replication rate. The data indicate the low-to-high inhibition (concentration-dependent) of parasite proliferation starting from the smallest concentrations of the Cu-II solutions. Both solutions' statistically significant antiproliferative activity was observed at the three highest concentrations ($P < 0.05$). Moreover, the statistical variance was also present between the highest concentrations compared to the lowest ones of both tested solutions. In addition, $CuSO_4$ presented a better effective antiparasitic activity than $CuCl_2$, despite the absence of statistical variance between the groups ($P > 0.05$). Our preliminary data show that Cu-II solutions were active against *T. cruzi* and can be considered a potential new antichagasic therapy. A new round of experiments is planned to provide host cell-safety data. **Keywords:**Metallotherapeutic agents;Antichagasic;Therapy.

PV-24 - **Deciphering the role of Orc1b in the activation of DNA replication in *Trypanosoma cruzi***

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Trypanosoma cruzi genome is high repetitive due the presence of rapidly evolving multigenic families that code for surface proteins that contribute the success of infection. DNA replication is the process that warrant genomic maintenance; however, incomplete, erroneous or premature DNA replication can generate mutations and SNPs, chromosomal aneuploidy or polyploidy, and gene copy number variation. Since the genomic plasticity plays a key role as T. cruzi infection success, it is important to dissect the molecular bases of DNA replication in this parasite. The duplication of the genome beginning in the G1 phase of the cell cycle when the Origin Recognition Complex (ORC), composed in eukaryotes by six subunits, is recruited onto replication origins. The composition of ORC in Trypanosoma, however, is still under discussion. Four proteins were described as ORC components, Orc1Cdc6, 7980, 3120, and ORC4. In Trypanosoma brucei, Orc1b was identified as a Orc1Cdc6 interactor, involved at DNA replication, but that interacts with ORC only during S stage of the cell cycle. Here, we performed the Chip-seq methodology using anti-TY1 in a T. cruzi lineage containing tagged Orc1b. The Orc1b localization was analyzed in relation to previously data from our group that include Orc1Cdc6 localization and active (Orc1Cdc6 sites that was fired) and inactive (not fired Orc1Cdc6 sites) origins. We found that Orc1b co-localizes with Orc1Cdc6. Moreover, while Orc1b in found at Orc1Cdc6 sites and upstream Orc1Cdc6 in inactive origins, it was found only upstream Orc1Cdc6 sites at active ones. These data suggest that Orc1b interacts with ORC even in non-active origins and is dislocated during origin activation. The presence of Orc1b in dormant origins raises the discussion concerning the role of Orc1b in origin activation proposed for T. brucei. therefore we are now investigating the possibility of Orc1b replaces Orc1Cdc6 in some DNA regions. **Supported by:**2020/00694-6 Fapesp **Keywords:**Trypanosoma cruzi;DNA replication;Origin Recognition Complex.

PV-25 - **Iron Storage in *Trypanosoma cruzi***

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Fe is an essential cofactor in many metabolic pathways. The parasite *Trypanosoma cruzi* causes Chagas' Disease, and heme and ionic Fe are required for optimal growth, differentiation, and invasion. Fe is also harmful due to catalyzing the formation of reactive O_2 species; for this reason, all living systems develop mechanisms to control the uptake, metabolism, and storage of Fe. Two main proteins — a Fe-reductase, necessary for reducing Fe^{2+} of the Fe^{3+}, and an iron transporter, which takes up Fe^{2+} as substrate — are involved in the ionic Fe transport through *T. cruzi* plasma membrane. Once in the cytosol, free Fe must be stored or metabolized to avoid the production of free radicals. Bloodstream forms of *T. cruzi* contain iron-rich acidocalcisomes, indicating that this organelle could be responsible for Fe storage. Although free Fe was not observed in acidocalcisomes in other evolutionary forms, this cannot rule out the possibility that in cultured cells, Fe supplementation causes acidocalcisomes of different evolutionary forms to have different functions or storage resources. In plants and *Saccharomyces cerevisiae*, specific membrane proteins transport iron (VIT1/FPN2/Ccc1) into vacuole storage. Here, we found a putative vacuolar iron transporter sequence: it was found in the genome database of *T. cruzi* (TriTrypDB: BCY84_12276) following a BLAST search using the Fe transporter TbVIT from *T. brucei* (TritrypDB: Tb927.4.4960) as a target. This VIT1 ortholog in *T. cruzi*, TcVIT, could be responsible for Fe storage in *T. cruzi*. The deduced amino-acid sequence of the peptide comprises 469 residues, thus resulting in a predicted molecular mass of 50.6 kDa. The deduced protein TcVIT has five possible transmembrane domains and a highly conserved domain corresponding to the vacuolar metal transporter (VIT1/CCC1) family domain. The localization of this vacuolar iron transporter on acidocalcisomes could indicate the role of this organelle in iron storage. **Keywords:** Fe storage;acidocalcisome; trypanosomatids.

PV-26 - **DESIPHERING THE *TRYPANOSOMA CRUZI* HIGH MOBILITY GROUP B PROTEIN (TcHMGB) ROLE IN GENE TRANSCRIPTION**

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High Mobility Group Bs (HMGBs) are abundant non-histone chromatin proteins that contribute to chromatin organization and function, impacting various cellular processes including gene expression, DNA replication, repair, and recombination. Through interactions with DNA and other proteins they can influence the accessibility of genes and the assembly of transcriptional complexes. *Trypanosoma cruzi* HMGB (TcHMGB) is expressed in the nucleus in all the parasite life stages and can alter chromatin structure like its mammalian orthologs. Given the unique characteristics of transcription in trypanosomatids and evidence of epigenetic mechanisms controlling gene expression, our hypothesis is that TcHMGB may have a key role in transcription control.
With the aim of investigating TcHMGB functions, we first constructed transgenic parasites capable of overexpressing the protein under tetracycline induction, which showed that *Tc*HMGB can alter chromatin DNA structure making it more sensible to micrococcal nuclease treatment. *Tc*HMGB overexpression affected the parasite fitness, caused a dramatic decrease in epimastigote- and amastigote-proliferation, presumably impairing cell division and lowered trypomastigote infectivity *in vitro*. Then, we used CRISPR/Cas9 to generate knockout (KO) mutants. We verified gene edition by PCR and lower TcHMGB expression by qRT-PCR and western blot in transient transfectants. However, despite multiple attempts, we were unable to generate stable *TcHMGB* KOs, which suggests TcHMGB gene is essential or crucial for the parasite´s normal functioning.
Finally, to study TcHMGB role on transcription, we induced its overexpresion on epimastigotes and analized the RNA content using different approaches: labeling and detection of nascent RNAs; qRT-PCR and RNA-seq. Our results suggest that although some genes showed differential expression in overexpressing parasites, TcHMGB seems to have more global effects. **Supported by:**AGENCIA I+D+I, PICT 2019-4212;.CONICET, PIP 2021-0848 **Keywords:**TRYPANOSOMA;HIGH MOBILITY GROUP B;TRANSCRIPTION.

PV-27 - **Changes in chromatin accessibility reveal opposing patterns between tRNA genes with most genomic compartments during the *T. cruzi* differentiation.**

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The tRNA genes (tDNAs) contain the genetic information for transfer RNA (tRNAs) expression, which function as an adapter molecule linking a specific amino acid to its corresponding mRNA codon during the protein synthesis. *T. cruzi,* the etiologic agent of Chagas Disease, has a complex life cycle with different life forms living in vertebrate or invertebrate hosts. Recently, we detected by the FAIRE-seq analysis that the open chromatin regions are more enriched in epimastigote (Epi) than in metacyclic trypomastigote (MT) forms mainly at tDNAs. Interestingly, the differentiation of Epi to MT life forms is followed by a decrease in their translatome and transcriptome. We hypothesized that the closed tDNAs chromatin observed in MT might be associated with these events and could play a key role in parasite differentiation. Therefore, this work aimed to analyze whether the tDNA chromatin changes are essential to regulate tRNA expression and to determine the moment, during metacyclogenesis, that tDNA's chromatin became closed. Thus, we applied the FAIRE-seq technique during the epimastigote to metacyclic trypomastigote differentiation. Our results revealed an overall increase in tDNAs open chromatin upon 24 hours of parasite differentiation in TAU 3 AAG medium. In contrast, the open chromatin profile decrease at other loci, such as those from protein-coding genes and interpolicistronic regions. These findings are intriguing and suggest a higher tRNA transcriptional activity in epimastigotes incubated in TAU 3 AAG for 24 hours than in epimastigotes in the exponential phase. Currently, we are performing tRNA-seq and ChIP-seq to respectively measure mature tRNA expression and quantify the presence of RNAP III machinery in the tDNAs during parasite differentiation. These data will be essential for understanding the role of tDNA chromatin alterations and parasite differentiation. **Supported by:**FAPESP 21/11419-9 e FAPESP 18/15553-9 **Keywords:**Trypanosoma cruzi;tDNA;cell differentiation.

PV-28 - **THE PROFILE OF RESEARCH IN TRITRYPS BETWEEN 2010 AND 2021: A SYSTEMATIC REVIEW**

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Leishmaniasis, Chagas disease and sleeping sickness (TriTryp diseases) are caused by the parasites Leishmania spp., *Trypanosoma cruzi* and Trypanosoma brucei, respectively. These infections represent a global public health concern, particularly in tropical regions of South America, Asia and Africa. The aim of our study was to systematically evaluate publications of TriTryp articles over the last 12 years, with a special focus on Brazil, historically a prolific producer of Trytrips articles due to the prevalence of the disease and the studies by Carlos Chagas developed in the early 20th century. Three comprehensive searches were performed on Medline-PubMed to collect publications from January 1, 2010 to December 31, 2021. Using the MeSH terms: leishmania or leishmaniasis; brucei, Trypanosoma or African sleeping sickness; *Trypanosoma cruzi* or Chagas. Data analyzes were performed using the R programming language (version 4.2.1). For the analysis, 6,478 articles were included, with Brazil contributing an average of 27.5% of the publications. Brazilian publications increased from 405 to 726 per year in the last decade. Regional disparities were observed, with the Southeast region leading and the Northeast/North regions with lower averages. The Northeast presented a growing index of publications. Leishmania was the most studied parasite in Brazil. Original articles predominated, mainly in the Southeast region. International collaborations were associated with greater quantity and impact of publications. The Southeast had the highest impact factor, while the Northeast showed significant growth. This study emphasizes Brazil's importance in TriTryp research, highlighting increased publications in the Northeast and North, particularly on leishmaniasis compared to Chagas disease. Regional variations in publication rates, publication type, parasite focus, collaboration, and impact underscore the need for ongoing research to address these public health challenges. **Supported by:**FAPEMIG, CNPq, CAPES. **Keywords:**TriTryps;Manuscripts Evaluation;Regional Development.

PV-29 - **Molecular and structural characterization of *Trypanosoma cruzi* flagellar pocket cytoskeleton**

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Trypanosoma cruzi is a flagellated protozoan of the *Trypanosomatidae* family that displays a **flagellar pocket structure (FP)**, an invagination of the plasma membrane from which the flagellum emerges. Significant studies highlight the FP as an important site of endo/exocytosis, which contributes to the parasite's different survival mechanisms. In *T. brucei* trypomastigotes, several FP structural proteins were described with important function in cell morphogenesis and viability, as TbBILBO1, TbMORN1, TbFPC4 and TbSPEF1. Among these, TbBILBO1 stands out for being essential for FP formation and it was localized exclusively in the FP collar. Genomic data suggested the existence of orthologs for these proteins in *T. cruzi*, in which the FP remodeling is important during differentiation in the three developmental forms: epimastigotes, trypomastigotes and amastigotes. Using CRISPR-Cas9 technique, we generated epimastigote cell lines expressing TcMORN1, TcFPC4 and TcSPEF1 tagged with mNeonGreen (mNG) tag. Immunofluorescence assays using anti-BILBO1 antibody indicated that the protein is not located exclusively in the flagellar pocket collar of *T. cruzi* epimastigotes, but also in a projection that extends into the interior of the cell body. Negative staining of cytoskeleton fractions showed the participation of BILBO1 in the cytostome-cytopharynx, a structure related to endocytic processes in *T. cruzi* epimastigotes. TcBILBO1 exhibited a partial colocalization with TcMORN1, which encircles the flagellum exit site. Our data strongly suggests that BILBO1 and MORN1 organizations are different between *T. brucei* trypomastigotes and *T. cruzi* epimastigotes and this may be related to different morphology and ecological niche. Subcellular and relative localizations and ultrastructural assays are being performed with the other tagged proteins in all forms of *T. cruzi* to better describe the molecular and structural organization of the flagellar pocket. **Supported by:**FAPERJ - 201.908/2022 **Keywords:**Trypanosoma cruzi;flagellar pocket;cytoskeleton.

PV-30 - **CHARACTERIZATION OF CRK1 KINASE IN THE CONTROL OF CELL PROLIFERATION AND METACYCLOGENESIS OF *Trypanosoma cruzi***

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Cyclin-dependent kinases (CDKs) are essential proteins that regulate the cell cycle in eukaryotes, and their modulation depends on environmental and mitogenic factors. In *Trypanosoma cruzi*, the causative agent of Chagas disease, different evolutionary forms can be found in its hosts, differing in terms of morphology, metabolic profile, and replicative capacity. The transition between replicative and infective forms depends on different factors such as nutritional stress and osmolarity changes, which can lead to progression, quiescence, and cellular differentiation. Considering the importance of proliferative control for the life cycle of *T. cruzi*, the present study aimed to investigate and characterize the CRK1 kinase (cdc2 related kinase), a homolog of CDK1, throughout the cell cycle and in the metacyclogenesis of the parasite. To this end, the CRISPR/Cas9 methodology was used to obtain a *T. cruzi* lineage (Dm28c Cas9) expressing the protein tagged with NeonGreen. Through hydroxyurea (HU) synchronization, metacyclogenesis, and immunofluorescence techniques, it was possible to evaluate the levels of CRK1 expression in the cell cycle and metacyclogenesis, as well as its localization. The results demonstrated that the CRK1 kinase is constitutively expressed throughout the cell cycle of *T. cruzi*. On the other hand, the kinase shows reduced expression in metacyclic trypomastigote forms. Regarding its localization, preliminary results suggest a cytoplasmic localization and no nuclear localization at any time. Furthermore, CRK1 appears to have a mitochondrial localization pattern in G1/S and G2 epimastigotes. Therefore, it is considered that the dynamics of CRK1, in terms of its expression and localization, do not differ from what has been described in the literature for CDKs in other eukaryotes. **Supported by:**FAPESP_2022/08866-6 **Keywords:**CRK1;Cell cycle;Trypanosoma cruzi.

PV-31 - **Participation of the ABCA1 transporter in the cholesterol traffic in *Trypanosoma cruzi*.**

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Trypanosoma cruzi presents different developmental forms according to host where the parasite is found. The epimastigote forms are found in the digestive tract of triatomines and presents high rates of endocytic activity, absorbing molecules such as LDL (low-density lipoprotein). The uptake of LDL (cholesterol) pass through the endocytic route up to the reservosomes, where cholesterol is stored or distributed to the rest of the cell according cell demand. Part of cholesterol goes to lipid droplets (Pereira et al, 2018). The absorption, exit and distribution of cholesterol is well stablished in higher eukaryotic models. However, there is little information about cholesterol traffic in pathogenic protozoans, including T. cruzi. The ABCA1 transporter (from the ABC transporter family) participates in the reverse cholesterol transport in mammals, and it was also described in flagellar pocket membrane and reservosomes of epimastigotes (Torres et al, 2004). However, its role continues unexplored in T. cruzi. Our aim is to understand how the TcABCA1 transporter contributes to the cholesterol traffic in epimastigotes and its involvement in cholesterol exit from reservosomes to other subcellular compartments. Besides, the description of TcABCA1 in flagellar pocket opens questions about the cholesterol efflux in the parasite. After incubation of epimastigotes in medium supplemented with ABCA drug inhibitors as Cyclosporin A (0 - 20 uM) or DIDs (0- 200 uM), we observed almost 85% of reduction of LDL - TopFluorCholesterol uptake and storage in reservossomes. The parasite viability and enzymatic metabolism were assessed by MTS/PMS assay. We produced mutant parasites that expressed TcABCA1-mNeonGreen and observed the addressing to the reservosomes. Our strategy now is to produce knockout parasites TcABCA1 (-/-) in order to study the implications in proliferation and viability, LDL traffic from reservosomes to other compartments or mobilization of neutral lipids from lipid droplets. **Supported by:**FAPERJ 205.229/2022 **Keywords:**Trypanosoma cruzi;Cholesterol traffic;ABCA1 transporter.

PV-32 - **Use of the CRISPR/Cas9 to evaluate the Localization and Expression of the Cyclin 5 in epimastigotes of *Trypanosoma cruzi* throughout the Cell Cycle**

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Trypanosoma cruzi, the etiologic agent of Chagas Disease, has a complex life cycle composed of different life forms (present in invertebrate or vertebrate hosts) that vary about their replicate capacity, where infective forms do not replicate. In model eukaryotes, the replication control occurs throughout the cell cycle and is regulated by cyclin-dependent kinases (CDKs) and cyclins. In *T. cruzi*, cyclin 5 stands out, a kinase-activating protein that interacts with CRKs (homologous proteins of CDKs) 1 and 3. In addition, it presents modulations in its phosphorylation sites during the change from the epimastigotes to the metacyclic trypomastigotes forms, a process known as metacyclogenesis. Given this, cyclin 5 can be a promising target in understanding the proliferation and differentiation processes of the parasite. Thus, this work aims to characterize the cyclin 5, by generating a cyclin5-tag lineage and analysing its expression profile and location throughout the cell cycle of *T. cruzi*. For this, the CRISPR/Cas9 reverse genetics technique was used in epimastigotes to add tags Myc and Neon Green in the 5' portion of this gene of interest; and different assays were performed to validate the recombinant lineage and the protein expression and localization profile. The results showed that it was possible to generate and validate the CRISPR/Cas9 modification in the N-terminal region of the cyclin 5 protein, giving rise to the blasticidin-resistant strain cyclin5_Myc_NG. Cyclin 5 showed a possible differential expression, with higher expression in the S phase, and cytoplasmic localization, with evidence of clusters in the region around the nucleus and kinetoplast. However, further analyzes of cluster patterning and expression are being performed to validate our previous data. Obtaining the lineage allowed us to carry out further studies of localization and expression in other life forms of the parasite, in addition to the epimastigote. **Supported by:**FAPESP/ 2022/02243-7 **Keywords:**Cyclin 5;Cell Cycle ;Trypanosoma cruzi.

PV-33 - **Role of NADPH-oxidase Inhibitors in *Leishmania amazonensis* LFR1 Activities**

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Leishmania amazonensis occurs in several parts of Brazil and is the causative agente of cutaneous leishmaniasis. The parasites are transmitted by sandflies insect vectors to vertebrate hosts in the flagellated form called metacyclic promastigote. In the vertebrate host, promastigotes differentiate into aflagellated intracellular amastigotes and begin to multiply within macrophages. Recently, our group showed that heme, an essential nutrient that *Leishmania* needs to acquire from the host, activates a ferric reductase enzyme (LFR1) that has also a NADPH-oxidase (NOX) activity with high production of hydrogen peroxide (H_2O_2), and crucial importance to the differentiation of the parasite to amastigote forms. NOX enzymes are involved in several pathologies such as cancer, heart disease and neurodegenerative diseases. Therefore, there is an intense search for drugs that inhibit the activity of these molecules. Some of these inhibitors are commercially available, such as GKT137831 (Setanaxib), and GKT136901. In this context, the objective of this study is to investigate the effect of these NOX inhibitors on both LFR1 activities of *L. amazonensis* and to verify the effect of these drugs on parasites proliferation and differentiation. Preliminary results using MTT cytotoxicity test, show that the tested compounds were not able to cause cytotoxity on *L. amazonensis* after 24 hours of treatment. However, both were able to inhibit LFR1-NOX activity in a dose-dependent manner. It is possible that inhibition of LFR1 by GKT137831 and GKT136901 is involved in the differentiation of the parasite to the amastigote forms, rather than in the cytotoxicity of the promastigote forms. We are still evaluating toxic effects for longer periods of time. Also, we do not rule out the possible toxicity to amastigote forms. These and other investigations are on going in our laboratory. **Supported by:**FAPERJ Processo 211.283/2019 **Keywords:**Setanaxib;GKT136901;Ferric-reductase.

PV-34 - **The route to go: Identifying key amino acid metabolic steps as potential drug targets for Chagas disease treatment using bar-seq CRISPR-Cas9**

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Trypanosoma cruzi, the causative agent of Chagas disease, undergoes a complex digenetic life cycle, which entails environmental changes that result in substantial variations in temperature, osmolarity, and nutrient availability. Within this context, amino acids and their metabolism play crucial roles in cell differentiation, maintenance of cell volume, and response to diverse forms of stress. This study aims to identify the key steps within the amino acid metabolic network that are essential for *T. cruzi* to complete its life cycle. To achieve this goal, we are employing the bar-seq CRISPR-Cas9 genome editing strategy to generate mutant knockout cell lines for 40 enzymes that are putatively involved in amino acid metabolism. Each cell line is assigned a unique bar-code sequence, and they will be pooled together to facilitate parallel phenotyping during cell differentiation and infection, both *in vitro* and *in vivo*, employing next-generation sequencing. The relative fitness throughout the various stages of the life cycle will be assessed by quantifying barcode abundance, enabling the identification of enzymes that are vital for parasite development and infectivity. Thus far, we have successfully generated 24 mutant cell lines, comprising 12 hemi-knockouts and 12 total knockouts. Interestingly, the enzymes for which only partial knockout of the coding sequence was attainable are associated with Asn, Asp, Ser, and Thr metabolism, while complete knockout was achieved for enzymes involved in Glu, Ala, Met, Trp, and Gly metabolism. This strategy will provide insights into the metabolic pathways that *T. cruzi* has evolved to adapt to diverse environments and will also yield novel targets for future drug design. **Supported by:**FAPESP Proc n.o. 2021/12938-0, Welcome Trust Proc n.o. 222986/Z/21/Z **Keywords:**Trypanosoma cruzi;CRISPR-Cas9;Amino acids.

PV-35 - **Synthetic Biology in the Study of Protein Acetylation in Protozoan Parasites**

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Protein acetylation is present in histones and non-histones proteins and several works have been demonstrating the impact of this modification in several biological processes. Our group demonstrated the protein acetylated profile of *Trypanosoma cruzi* and *Trypanosoma brucei* and found several glycolytic and antioxidant enzymes acetylated in both parasites. Also, we validated the impact of this modification in the enzymatic activity of aldose, a glycolytic protein, and superoxide dismutase A (SODA), a key protein in the oxidative stress response. To gain insight into the role of protein acetylation in trypanosomatids we decided to establish a synthetic biology system based on the orthogonal pair pyrrolysyl-tRNA synthetase and the tRNApyl, derived from the *Methanosarcina barkeri* archaea specie, which allows the insertion of an acetylated lysine into a specific position of the protein for further biochemical analyses. To validate this system, we choose the *T. brucei* aldolase and SODA, and *T. cruzi* SODA, which was used to insert the amber codon at K157 for aldolase, and K66, K74, K97, and K101 for SODA, and cloned into pET28a vector. All the lysine sites were previously detected acetylated in our analyses. The orthogonal pair (pyrrolysyl-tRNA synthetase and the tRNApyl) together with the specific pET28a constructs were transformed in BL21 *E. coli*, and we successfully expressed the native and acetylated aldolase proteins. The acetylation of aldolase was confirmed using dot blot assays with the specific anti-acetyl-lysine antibody. In parallel, we also tested the expression of all versions of SODA acetylated at K66, K74, K97, and K101 sites. Experiments are in progress to test the enzymatic activity of acetylated aldolase and to purify the SODA proteins. In conclusion, we successfully established the orthogonal pair tool for the expression of acetylated proteins in bacteria, opening the opportunity to better understand the function of this modification in trypanosomatids **Supported by:**Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) – Processo 424729/2018-0 **Keywords:**Protein Acetylation;Synthetic Biology;Orthogonal pair.

PV-36 - **Studies of mitochondrial carriers in *L. major***

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Mitochondria are central organelles involved in many cellular processes. The inner mitochondrial membrane is impermeable to most solutes, which can only reach the matrix through the transport mediated by specific carriers. Mitochondrial carries display six transmembrane helixes with specific domains. *Leishmania* genus includes etiological agents of different forms of leishmaniasis. Most of mitochondrial carriers display low similarity to human counterparts (around 40%), representing targets for future drug design. Then we aimed to investigate if *L. major* displayed mitochondrial carriers, through bioinformatic and metabolic assessment of mitochondrial carriers in *L. major*.
Based on *Sacharomyces cerevisiae* sequences and using BLASTp algorithm we search for *L. major* sequences limiting Tritryp score to 50. From these sequences that were found for *L. major*, phylogenetic trees of maximum parsimony were constructed using Mega X program with yeast and human carriers. Based on ortology informations a table was constructed listing potential *L. major* mitochondrial carriers. A total of 37 candidates were found, expanding the previously reported inventory of mitochondrial carriers in *Leishmania*. Using ScanProsite tool conserved domains was investigated. Using online tools: Target P, Predotar and MULocDeep mitochondrial addressing was inspected. Additionally, most of the carriers were found in the proteome (TriTrypDB database), providing protein level evidence. Finally, respirometry analyses performed on intact parasites revealed that N-ethylmaleimide, a classical and non-specific inhibitor of mitochondrial phosphate carrier, significantly reduced oxygen consumption. This strongly indicates the existence of a functional mitochondrial phosphate carrier in *L. major* parasites. Based in all of these evidences we observed that *L. major* present putative mitochondrial carriers, that must be further investigated. **Supported by:**POM/FIOCRUZ e PAEF/FIOCRUZ (Lab. de Pesquisa em Leishmanioses, IOC, FIOCRUZ, Rio de Janeiro, RJ, Brazil), CNPq (308629/2021-3), FAPERJ (E-26/210409/2019).
Keywords:LEISHMANIA;PARASITIC BIOCHEMISTRY;MITOCHONDRIAL CARRIERS.

PV-37 - **Single knockout of bioenergetic enzymes leads to metabolic changes in*Trypanossoma cruzi* epimastigotes cells.**

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Trypanosoma cruzi, the pathogen responsible for Chagas disease, is transmitted by triatomine insects during their blood-feeding. Heme, a byproduct of blood digestion, plays a crucial role as a physiological oxidant molecule. It not only stimulates the proliferation of epimastigotes (a proliferative stage of the parasite) but also regulates the expression of genes associated with energy metabolism. Specifically, heme has been observed to upregulate genes involved in glycolysis and aerobic fermentation processes. Given the significance of these metabolic pathways in meeting the energy demands during this proliferative stage, this study aims to investigate the impact of genetic manipulation using the CRISPR/Cas9 system on genes related to energy metabolism in *T. cruzi* epimastigote. The gene knockout was validated by PCR and showed that only parasites lines single knockouts were obtained: glycosomal malate dehydrogenase (MDHg), glycosomal hexokinase (HKg) and cytoplasmic malic enzyme (MEc), suggesting that these genes can be essential for *T. cruzi* epimastigotes. Then, proliferation of these single knockout mutants was evaluated to determine whether it had an impact compared with both the control group, where the genes were not deleted, and the wild type cells. There was a reduction of approximately 65.7% in proliferation after seven days for the mutant of the MDHg enzyme. When conducting a proliferation curve, it was observed that the mutant showed no growth until day three, and between days four and five, it proliferated but at a rate 80% lower than the control. On the other hand, there was no difference in proliferation after seven days for the HKg mutant. Proliferation was also evaluated in the presence of Mitotempo, a reagent that removes mitochondrial ROS, and 2-deoxyglucose (2-DG), a competitive inhibitor of hexokinase. We are studying the viability and metabolism of these mutants, predicting their ability to interact with their vector and transmit the disease. **Supported by:**FAPERJ, CAPES, CNPq and INCT-EM.
Keywords: Trypanosoma cruzi;heme;energy metabolism.

PV-38 - **Structural, phylogenetic and functional analysis of E3 ubiquitin-ligase CRLs (Cullin RING-ligases) genes of Leishmania infantum**

TORRES, C.; DE CORREIA, C.R.S.T.B.; ALVES, E.G.; RODRIGUEZ, G.M.; ROGERIO, L.; TAKAMIYA, N.T.; MARUYAMA, S.R.C.; TEIXEIRA, F.R..
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The ubiquitin proteasome system (UPS) is responsible for intracellular proteolysis in eukaryotes. E3 ligases play a key role in the ubiquitination process, targeting proteins for degradation by proteasome or processing by deubiquitinating enzymes. Cullin-RING ligases (CRL) are the largest and most studied class of E3 ligases in mammals. They are composed by SKP1, Cullin 1, RBX1 and an F-box protein that interacts with SKP1 through the F-box domain and recruits substrates. In parasitic protozoans with host alternation in their life cycles, intracellular proteolysis is essential for parasitism. Little is known about UPS in many parasites, including trypanosomatids of the genus Leishmania, responsible for causing leishmaniasis. Previous results from our group demonstrated that the CRL complex exists in L. infantum and the interactions between its components were validated. Here we performed in silico phylogenetic and structural analysis of L. infantum genes orthologs to the human SKP1, CUL1 and RBX1. We observed that L. infantum proteins are structurally similar to the human proteins with conserved interaction motifs among the CRLs proteins compared to their orthologs in H. sapiens. Phylogenetic analysis of orthologous proteins from 12 trypanosomatids showed that L. infantum proteins are more related to L. donovani BPK282A1 proteins, as expected. In parallel, SKP1 and Cullin1 interactome mass spectrometry of L. infantum protein extracts revealed a nucleotide hydrolase associated to SKP1 protein. We successfully cloned it in mammalian cell expression plasmids and confirmed its expression in HEK293T transfected cells. Furthermore, co-immunoprecipitation in HEK293T cells together with SKP1 will be performed. Also, a strain of L. infantum expressing myc-SKP1 and HA-hydrolase will be generated by CRISPR/Cas9 in order to demonstrate their interaction in promastigotes. Thus, these results contributes to functional characterization of CRLs in L. infantum. **Supported by:**2022/16270-6
Keywords: Cullin-RING ligase;Bioinformatics;Leishmania inf.

PV-39 - **Construction of a recyclable and compact expression vector for *Trypanosoma cruzi***

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To overexpress exogenous genes in T. cruzi some expression vectors are available such as pTEX, pRIBOTEX, pTREX, pTcINDEX, pTcGW, and pROCK. Most of the expression vectors use neomycin resistance gene, followed by hygromycin resistance, and few reports use Blasticidin or puromycin resistance genes as selectable markers. Additionally, the empty expression vectors have 7-10 kb in size, which limits the size of the transgenes that can be cloned into them. This limited number of selectable markers used and the size of the vectors prompted us to develop a new series of vectors. Aiming to construct the vectors easily, we have adapted the Biobrick strategy based on T. cruzi genome features creating the TcBIOBRICK. In the Biobrick approach, each plasmid segment is called a brick, which can be joined by using only 4 restriction enzymes including compatible end enzymes. Several segments can be assembled using the same enzyme set. Using the TcBIOBRICK approach, we build pTcMini-NG, a 5.5 kb vector for stable expression including ribosomal promoter, the mNeon Green as reporter cassette, and NeoR gene flanked by loxP sites. pTcMini-NG was stably transfected into epimastigotes, and the NG-positive parasites population was reverted to G418-sensitivity by CRE editing, i. e. by electroporation with recombinant CRE recombinase. **Supported by:**Fundação Araucária **Keywords:**Biobrick;pTcMini;plasmid.

PV-40 - **Exploring *Trypanosoma cruzi* sirtuins as therapeutic targets in Chagas disease**

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Chagas disease, also known as American trypanosomiasis, is a parasitic disease caused by the protozoan *Trypanosoma cruzi*, and endemic in Latin America, affecting more than 7 million people. During its life cycle, *T. cruzi* transits between an invertebrate and a vertebrate host and has been exposed to environmental changes that require its adaptation to survive. Acetylation is a post-translational modification of proteins present in the *T. cruzi* parasite and is regulated by two classes of enzymes: lysine acetyltransferases, which adds the acetyl group, and lysine deacetylases that removes the acetyl group from lysine residues. Sirtuins are NAD+-dependent lysine deacetylases involved in several cellular processes and have been also explored as drug targets in several diseases. Previous work from our group characterized *T. cruzi* sirtuins (TcSir2RP1 and TcSir2RP3) and demonstrated the potential to explore these enzymes as potential drug targets in this parasite. To advance in the validation of *T. cruzi* sirtuins as drug targets we used the TcSir2rp1 and rp3 purified heterologous proteins to test several biochemical parameters important for deacetylation activity, including protein concentration, reaction time, temperature, pH, substrate and NAD+ concentration. We found that in general, both enzymes had similar biochemical parameters, except for Km, Vmax and Kcat, while TcSir2rp1 has a Km=42,46 µM, Vmax=0,0170 µM.s and Kcat=0,0282 s^{-1}; TcSir2rp3 has a Km=74,11 µM, Vmax=0,0169 µM.s and Kcat=0,01 s^{-1}. Using the best deacetylation conditions we tested the inhibitory capability of a library of 23 SIRT inhibitors against TcSir2rp11 and rp3. These inhibitors were designed based on the chemical structure of previous SIRTi tested in our lab and we found that 3 and 5 compounds presented >35% of inhibitory activity, against TcSir2rp1 and rp3, respectively. Experiments are in progress to determine de IC50 of each compound and to test their effect on *T. cruzi in vitro* infection. **Supported by:**CAPES - 88887.64746/2021-00
Keywords:T; cruzi ;Sirtuins;Chagas disease.

PV-41 - **Cell division-associated targets are modulated in kharon1-deficient *Leishmania infantum***

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The disruption of *kharon1* gene in *Leishmania* spp. leads to live-attenuated amastigotes, impairing cell cycle progression due to cytokinesis defect, making the parasites unable to maintain the infection, which can be used to better understand the molecular basis of cell division. Here we select *L. infantum kharon1* null mutants (Δ*Likh1*) by CRISPR/Cas9 and evaluate by RT-qPCR the modulation of transcripts that encode for 11 cell division/cytoskeleton-associated proteins: Centrin4, Sfi-1, SAS-6, KKT, KMP-11, AIRK, KHAP1 and KHAP2, KPP1, TFK1 and NUP92. In Δ*Likh1*, Sfi1, KHAP2, TFK1, and KPP1 were upregulated in promastigotes, while amastigote forms presented increased levels of mRNA that encodes to KHAP1 and KHAP2. In order to globally evaluate the transcript modulation, we performed cDNA sequencing of Δ*Likh1*. This resulted in the identification of 10 downregulated and 71 upregulated transcripts. Among them, we selected 5 upregulated targets that were associated with cytoskeleton and cell division process (SET/SET7, PK53, COP, AP2 and CYC6). They mRNA level was validated by RT-qPCR, whose upregulation was confirmed in promastigote forms, Furthermore, the PK53 encoding mRNA was upregulated in both, promastigote and intracellular amastigote forms. It may indicate compensatory actions, suggesting that during cell division kharon1 plays a role dependent on 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. Knowing that KHAP1 and KHAP2 were previously described as kharon-associated proteins 1 and 2 respectively, the subcellular localization of the proteins in promastigotes and amastigotes was performed, indicating corresponding locations of Kharon1. The basic findings here can be applied to better understand the mechanisms of chromosomal segregation and these proteins can be studied as drug targets for tackling leishmaniasis. **Supported by:**Fapemig, CAPES, CNPq
Keywords: Leishmania infantum;kharon1;cell division.

PV-42 - **Using *Trypanosoma brucei* to study *Trypanosoma cruzi* metabolism: the disturbance caused on *T. brucei* Pro consumption by expressing the *T. cruzi* His pathway insertion**

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The life cycle of *Trypanosoma cruzi* involves an invertebrate and a vertebrate host to completion, and this parasite is highly adapted to different environments. For example, when glucose is limited in the insect's gut, *T. cruzi* can consume amino acids producing ammonia. Histidine (His) is present in high concentrations in the insect's gut and the parasite can use it to generate ATP through its conversion to glutamate (Glu), a metabolite that can be produced by other sources, like Proline (Pro). This pathway consists of four enzymatic steps and coding sequences for the four enzymes are present in *T. cruzi*'s genome but not in *Trypanosoma brucei.* Aiming to investigate its biological role, we generated *T. brucei* procyclic cell lines able to express active versions of the first two enzymes of *T. cruzi*'s His degradation pathway. These lineages did not show differences in proliferation or ATP content in the presence of His as the solely metabolite of source compared with parasites in the absence of any metabolite (control). However, when Pro is used as the only metabolite, ATP levels were diminished when compared to wild-type controls. Additionally, O_2 consumption triggered by Pro diminished by 16% when compared to the control, while His did not affect O_2 consumption. The inner mitochondrial membrane potential did not show differences between wild-type and obtained lineages. We also obtained *T. brucei* cell lineages expressing the complete His degradation pathway which are under phenotypic analysis. Our findings using *T. brucei* as a scaffold for analyzing *T. cruzi* metabolic pathways are providing valuable insights into the functional implications of the *T. cruzi* histidine-glutamate pathway and its potential impact on parasite adaptation. **Supported by:**FAPESP - 2020/16569-6 **Keywords:**Trypanosoma cruzi;Histidine;Proline.

PV-43 - **Metabolic model curation for *Trypanosoma cruzi* and *Trypanosoma brucei***

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Genome-Scale Metabolic Models (GEMs) are computational tools that employ systems biology concepts to analyze the metabolic capabilities of organisms. These models combine genomic, biochemical, and physiological data to forecast metabolic behavior. Our objective was to create models for *Trypanosoma brucei* and *Trypanosoma cruzi* as tools to provide a descriptive and predictive outcome to enhance our comprehension of the metabolism of these important parasites. GEMs help to examine how metabolic networks are established around their constituent reactions and metabolites, facilitating the understanding of the subtle relations between reactions directly or indirectly related. Our models were made using COBRAToolBox and are now being manually curated. Curation involves in-depth research on the parasites' metabolism to identify genes and their related reactions, the main goal is to work on the amino acid metabolism for both models and to understand metabolic differences between *T. cruzi* and *T. brucei*. Our *T. cruzi* model has 559 reactions, 628 metabolites and our *T. brucei* model has 302 reactions and 497 metabolites. When evaluated for consistency, they showed values of 60% and 80%, respectively. Validation of these models involves simulating metabolic scenarios to generate predictions that can be experimentally confirmed. We will compare ATP productions and metabolite secretion from *in vitro* data to the model predictions and use this data to make the models more robust. To validate the *T. brucei* model we are generating *T. brucei* cell lines *in vitro* that express the histidine-glutamate pathway, which coding sequences for enzymes are present only in *T. cruzi's* genome. By comparing experimental results with model predictions, we aim to assess the impact of introducing a different pathway on metabolic flow. Analyzing the differences between these parasites will provide insights into their metabolism and adaptations to diverse environments. **Supported by:**FAPESP - 2020/16569-6
Keywords: Genome-Scale Metabolic Models;Trypanosoma;Curation.

PV-44 - **CHARACTERISATION OF A GENE ENCODING A TRYPSIN-TYPE SERINE PEPTIDASE IN *Trypanosoma cruzi* THAT PLAYS A ROLE IN METACYCLOGENESIS**

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In *Trypanosoma cruzi*, the differentiation of epimastigotes to infective metacyclic trypomastigotes occurs in the digestive system of the Triatomine bug upon nutritional stress. Parasite differentiation involves extensive remodelling both at the morphological and molecular levels, and proteolysis is thought to enable the degradation of epimastigote-specific proteins during this process. We have reported the identification of genes encoding an inhibitor of S1A-family trypsin-fold serine peptidases in the Tri-tryps, which contribute to the infectivity of *T. brucei*, *Leishmania sp*. and *T. cruzi*. However, no genes for putative S1A peptidases were identified in their genomes. Here, we describe the identification of a gene encoding a putative trypsin-type enzyme in *T. cruzi* (*TPS*), which is similar to trypsin-2 type serine peptidases present in bacteria. The alpha-fold model of the coding sequence predicted a protein with a N-terminal trypsin-type domain, followed by an alpha-helix rich region. To investigate its biological function, we generated *T. cruzi* Dm28 null-mutants (∆*tps*) and transgenic lines where the endogenous gene was fused to the mNeonGreen_Myc reporter using CRISPR/SpCas9. Western blotting of parasite lysates using antibodies to Myc showed that *TPS:mNeonGreen_MyC* is expressed in all life-stages. Fluorescence microscopy revealed that TPS is localised at the flagellum. ∆*tps* epimastigotes grow normally *in vitro*, but start to differentiate to metacyclic trypomastigotes prematurely (mid-log phase), while no significant alterations in the cell cycle were observed. Furthermore, at stationary phase ∆*tps* present higher percentages of metacyclic trypomastigotes as compared to the Dm28SpCas9 parental line. Tissue culture trypomastigotes ∆*tps* infect mammalian cell in vitro, but the intracellular burdens of amastigotes at 48hrs are slightly lower than that od the parental line. Collectively, our data suggests that TPS modulates parasite differentiation.
Supported by:FAPERJ e CNPq **Keywords:**Cruzi;Serine Peptidase ;Differentiation.

PV-45 - **MOLECULAR APPROACHES FOR CHARACTERIZING PROCESSING BODIES IN *TRYPANOSOMA CRUZI***

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Trypanosoma cruzi (Tc) is exposed to multiple environmental changes throughout its life cycle, demanding precise regulation of gene expression for the establishment of infection. In trypanosomatids, this regulation occurs predominantly at the post-transcriptional level, with mechanisms that stabilize messenger RNA (mRNA) playing a crucial role. Among these mechanisms, one may find processing bodies (PBs), complexes of RNA-binding proteins (RBPs) associated with untranslated mRNA that play a fundamental role in the storage and accessibility of the translation machinery to mRNA, and represent one of the most relevant mechanisms for the regulation of gene expression in Tc. TcDHH1, an RBP found in PBs, is widely used as a marker for this structure. Despite the intense investigation of the structural organization of Tc using various microscopy techniques, little is known about the ultrastructure of PBs. Therefore, this study aims to characterize the ultrastructure of PBs and investigate their interactions with other parasite organelles. We employed two main approaches: 1) generation of mutant parasites using the CRISPR/Cas9 system, and 2) electron microscopy analysis and correlative microscopy. Through PCR reactions, we amplified the SgRNA templates used in in vivo transcription and the donor DNA to generate labeled parasites (TcDHH1::mNG) or gene knockout parasites (TcDHH1-KO). After transfection with SgRNA templates and donor DNA for generating mutant parasites, we evaluated the fluorescence of the labeled parasites using flow cytometry and fluorescence microscopy, confirming the expression of the fluorescent protein with a similar localization reported in previous works using immunolocalization. The knockout parasites are undergoing selection. Subsequent steps include the validation of gene editing by PCR and Western blot. Electron microscopy characterization of the densities found by fluorescence microscopy is currently under development in our laboratory. **Supported by:**CNPq - 126068/2022-4
Keywords: Processing bodies;CRISPR-Cas9;Correlative microscopy.

PV-46 - **Is the Latitudinal Diversity Gradient applicable for unicellular eukaryotes? A Comparative Study of Arcellinida (Amoebozoa) in Brazilian Amazon and Temperate Mosses**

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The paradigm for microbial diversity is ‘Everything is everywhere, but the environment selects’. Moreover, the latitudinal diversity gradient (LDG) is a well established pattern of global species distribution. However, most microbial morphotypes (along with their ecology) have not been well characterized, and microbial diversity in the tropics is essentially unknown. Shelled amoebae form a paraphyletic free-living unicellular eukaryotic group that produces shells with taxonomic relevance. In this work in progress, we aim to compare the Arcellinida shell diversity of a moss sample from the pristine Amazon (Serra do Imeri) with the diversity found in mosses from the temperate regions. Alpha diversity, relative abundance and shell morphology of Arcellinida (Amoebozoa) will be analyzed for comparison. We identified taxa through the use of optical microscopy and scanning electron microscopy (SEM). Alpha diversity and relative abundance has been calculated with species curves. We compared the morphotypes found with previous taxonomic descriptions and SEM images. We found up to 3 times more Arcellinida taxons than in recent registers from mosses of temperate regions. More than 130 SEM pictures from different individuals were taken. Some shells of characteristic rare species (e.g. *Apodera vas* and *Certesella martiali*) showed phenotypical novelties. Based on our preliminary findings, we found evidence supporting the applicability of the LDG for the group in terms of species richness, however, some registers of rare morphotypes with novel phenotypes reinforces the perspective that molecular information from neotropical populations needs to be accessed to discuss if the lineages found in the tropics are the same as the registered in temperate zones.
Supported by:CNPq **Keywords:**Biogeographical comparison;Microbial diversity;Shell morphology.

PV-47 - **MAPPING THE *Trypanosoma cruzi* PROTEIN KINASE GENES UNIQUE AMONG THE TRI-TRYPS**

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Protein kinases (PK) are ubiquitous enzymes that transfer phosphate groups to proteins. In the pathogenic trypanosomatids, Trypanosoma brucei and Leishmania mexicana, kinome-wide screens identified several PK genes that are essential for parasite viability or required for infectivity in experimental models. Even though the sequence of the T. cruzi genome was made available nearly 20 years ago, the function of PKs has been underexplored in this organism. The putative function of PKs that are highly conserved among the tri-tryps could be potentially inferred in T. cruzi based on the phenotypes found in the PK-wide RNAi screen in T. brucei and the CRISPR/Cas9 genome-wide PK screen performed in L. mexicana. However, PK genes exclusive to T. cruzi have not been previously addressed. We performed a search in the available T. cruzi genomes for putative PK genes and identified approximately 41 genes that are absent from the available Leishmania genomes and 18 PK genes (PK1-18) that are absent from both Leishmania and T. brucei, and were thus considered T. cruzi unique. The 18 T. cruzi unique PKs are distributed among the STE, GMGC, and CAMK groups, and for one PK no matching group could be identified. Orthologues for those genes are present in a few other trypanosomatid species, e.g. T. conorhini, T. theileri, and T. melophagium, often sharing high sequence similarity. Structural modelling using alpha-fold predicted very high confidence for PK domains (>90% confidence) for 15 PKs while models were unavailable for 3 PKs. Seven T. cruzi unique PKs have orthologues in less than 5 Trypanosoma species. We have attempted to generate gene knockouts for those 7 genes, successfully generating null mutants for 3 and only heterozygotes for the other 4, suggesting their essentiality. Subcellular localisation was mapped by endogenous tagging using mNeonGreen_MyC as a reporter gene, revealing diverse distribution. Expression of the PKs in the parasite life stages is under investigation.
Supported by:faperj, cnpq, ukri **Keywords:**kinase;cruzi;function.

PV-48 - **Analysis of nascent transcripts in *Trypanosoma cruzi*.**

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Trypanosomatids have a genomic structure organized in polycistronic regions, and specific promoter sequences for each gene have not been described yet in *Trypanosoma cruzi*. Post-transcriptional mechanisms are the main ones responsible for regulating gene expression in these organisms. However, nascent transcription assays in specific genes indicate that some loci are transcribed at different rates. The present work aimed to understand the real impact of transcriptional regulation concerning the abundance of transcripts from different genomic regions using large-scale assays of nascent transcripts, known as *Global Run on sequencing* (GRO-seq). We first established a suitable computational pipeline to process and analyze nascent transcripts from different genomic regions of *T. cruzi*. Abundances were assessed by approaches based on coverages or on counting reads. Our data confirm that the GRO-seq data are enriched in nascent and unprocessed/degraded transcripts compared to the pool of mature RNAs detected by RNA-seq assays. It was observed that most PTUs have similar transcription rates. However, for some subsets of PTUs, significant differences were detected. PTUs and CDSs from the core genomic compartments are more abundant than those from the disruptive compartment. Furthermore, we detected a positive correlation between levels of chromatin opening and nascent expression, which is more evident when comparing genomic compartments. Taken together, our data point to a differential transcriptional regulation between genomic compartments associated with chromatin opening, suggesting an essential role for chromatin in the regulatory mechanisms of gene expression in *T. cruzi*. **Supported by:**Processo Fapesp 2018/15553-9 **Keywords:**Trypanosomatids;nascent transcription;computational pipeline.

PV-49 - **Biogeographic and temporal origin of Leishmania Ross, 1903 (Trypanosomatidae)**

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Several hypotheses have been proposed for the origin of *Leishmania*, based mainly on vector distribution and different hosts. The Neotropical hypothesis suggests the origin of *Leishmania* in the Neotropical region, and mammals would be their first vertebrate hosts. The Palearctic hypothesis places an origin in the Palearctic region and reptiles as their first vertebrate hosts. The Supercontinent hypothesis proposes an origin before the Gondwana breakup, with mammals as their first vertebrate hosts. The aim of the present study is to discuss the geographical origin of *Leishmania*, suggesting several processes that may have influenced current patterns of distribution of the genus based on hypotheses of phylogenetic relationships and methods of reconstructing ancestral distributional areas and molecular clock. Gene sequences from gGAPDH, HSP70 and V7V8 were retrieved from NCBI and TriTrypDB in a total of 54 terminal taxa. Genes were aligned on ClustalX and MAFFT7. Evolutionary models were accessed on jModelTest and Bayesian analyses were performed on MrBayes 3.2.7a. Reconstruction of ancestral areas was performed on RASP 4.2 using a Statistical Dispersal-Vicariance analysis. A dating analysis using relaxed molecular clock was performed on BEAST 2.5, with *Trypanosoma* as outgroup and data from *Paraleishmania* fossils. Bayesian analysis recovered *Leishmania* and its subgenera as monophyletic (pp=100%). S-DIVA analysis recovered as ancestral area of origin of *Leishmania* in the Neotropical region (p=35%), or Neotropical+Oriental region (p=34%) or Afrotropical+Neotropical+Oriental region (p=31%). Geographic events recovered by S-DIVA analysis corroborate the Supercontinent hypothesis. Initial molecular clock analyses show that the *Leishmania* parasites first appeared at about 80 mya, which suggests that they may have been originated before the Gondwana breakup. **Supported by:**CNPq
Keywords: Molecular Clock;Molecular Phylogeny;Biogeography.

PV-50 - **Sauroleishmania Ranque, 1973 emend. Saf'janova, 1982 (Leishmania: Trypanosomatidae): Bibliographic and phylogenetics review of the subgenus**

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Leishmania is a member of the subfamily Leishmaniinae and has three subgenera of medical and veterinary importance: *Leishmania*, *Viannia* and *Mundinia*. While those three subgenera infect mammals, *Sauroleishmania* is the only one described to infect reptiles. *Sauroleishmania* has a distribution restricted to the Old World and consists of 19 recognized species. These species are transmitted by sandflies of the genus *Sergentomyia* (Diptera: Psychodidae). The aim of the present study was to gather information in the literature about *Sauroleishmania* and to discuss the phylogenetic relationships between the species of this subgenus. Bibliographic data on *Sauroleishmania* were collected using several search tools, from its first citation to the most recent data. Sequences from HSP70 gene (619 bp) were retrieved from NCBI in a total of 45 terminal taxa (29 from *Leishmania*) and were aligned on ClustalX implemented on MEGA 10.1.8. Evolutionary model was accessed on IQ-Tree 2.2.2.6. The method of Maximum Likelihood was performed on MEGA 10.1.8 with 50 replications and GTR+I+G model. Maximum Likelihood analysis recovered *Sauroleishmania* as monophyletic, with 100% support and forming a sister group with the subgenus *Leishmania* (96%). Our study also used sequences from three undescribed lineages that cause visceral leishmaniasis in a province of China, and they were recovered in the same clade as other species of *Sauroleishmania*. Furthermore, only four species of the 19 subgenera of *Sauroleishmania* possess additional information beyond the initial description. For example, *L. (S.) henrici* has only one article from 1918 and none type material deposited in collections. The subgenus has many questions and little information about its species, both in the literature and in molecular databases. *Sauroleishmania* is composed of non-pathogenic species for mammals, which makes them an important model for molecular and evolutionary studies on the origins of *Leishmania* pathogenic species. **Keywords:**Leishmania (S;) henrici;Reptiles;Molecular Phylogeny.

PV-51 - **Disrupting Ubiquinone Biosynthesis in Malaria Parasites: a new strategy to amplify the effects of atovaquone**

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One of the most promising targets for malaria therapy development is the biosynthesis of isoprenoids. There are several metabolites that are important for *P. falciparum* and that are isoprenoids, but this study concentrates on the metabolism of only one of them: ubiquinone (or coenzyme Q; UQ). One of the most recently discovered antimalarials, atovaquone (ATO), is an inhibitor of mitochondrial complex III by competing with the reduced form of UQ, ubiquinol (UQH_2). This process shows that the availability of UQ/UQH_2 is critical for parasite respiration, which unlike in the human host, is precisely essential for pyrimidine biosynthesis. Therefore, this work aimed to evaluate the importance of the UQ biosynthesis pathway in the parasite by using inhibitors and creating an inducible knockdown line of PfCOQ2, predicted to codify a 4-hydroxybenzoate polyprenyl transferase (4-HPT), the first step of the UQ biosynthesis pathway. The reduced gene expression was found to affect the parasites' long-term viability and trigger increased susceptibility to ATO. Analogues of 4-hydroxybenzoate (4-HB) demonstrated UQ biosynthesis inhibitory action, specifically affecting the recombinant 4-HPT activity and potentiating the ATO antiplasmodial effect. Finally, it was possible to propose that alterations in the radicals linked to the C4 atom of the 4-hydroxybenzoate phenyl ring are more efficient than those linked to the carboxylic group in promoting the potentiating effects of ATO. These results open the way for the development of an antimalarial pharmaceutical formulation containing ATO that is more efficient than the one currently used. **Supported by:**FAPESP 2022/09526-4
Keywords: Malaria;atovaquona;ubiquinona.

PV-52 - **IDENTIFICATION OF A *Trypanosoma cruzi* PROTEIN KINASE GENE THAT PLAYS A ROLE IN METACYCLOGENESIS**

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Protein kinases (PKs) exert fundamental roles in cells, including signalling, replication, and differentiation. In the pathogenic trypanosomatids, Trypanosoma brucei and Leishmania mexicana, genome-wide screens revealed several essential PK genes and other PKs required for infection. In the genome of T. cruzi, we have identified eighteen putative PK genes (PK1 to PK18) that are absent from T. brucei and Leishmania, and thus were considered T. cruzi unique. PK5 was predicted to be from the STE group, and orthologues are present in T. rangeli, T. cruzi marinkellei (infects bats), T. conorhini (infects rats), T. theileri (infects bovines), and in T. melophagium (infects sheep). To investigate its function, we have generated null mutants (Δpk5) in T. cruzi Dm28 epimastigotes, as well as transgenic lines where the endogenous PK5 was fused at the C-terminus with mNeonGreen_MyC, using CRISPR/SpCas9. The expression of PK5:mNeonGreen_MyC was evaluated through Western blot, showing that it is expressed in epimastigotes but it is not expressed in tissue culture trypomastigotes. In epimastigotes, the protein has punctate distribution throughout the cell body. Δpk5 epimastigotes grew normally in vitro, but were unable to differentiate spontaneously to metacyclic trypomastigotes during stationary phase (up to day 12), in contrast with the SpCas9 parental Dm28 line. After 28 days, elongated forms were observed in Δpk5 cultures, which were resistant to lysis by human serum, suggesting that PK5 is required for timely differentiation. **Supported by:**Faperj, CNPq e UKRI
Keywords: Kinase;Cruzi;Mtetacyclogenesis.

PV-53 - **The knockout of *Leishmania braziliensis* Arginine Methyltransferase 5 alters the transcriptome of metacyclic promatigote forms and reduces amastigogenesis *in vitro***

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Leishmania braziliensis is the major causative agent of tegumentary leishmaniasis in South America. During its life cycle, the parasite alternates between several morphologies, most remarkably procyclic and metacyclic (META) promatigote forms (in the phlebotomine) and the amastigote (AMA) form (in the mammalian host). An efficient transition from META to AMA is particularly important for the establishment of the infection in the host cell. It was recently demonstrated in our lab that the knockout (KO) of the Arginine Methyltransferase 5 (*Lbr*PRMT5) in *L. braziliensis* reduces its ability to infect macrophages and to undergo amastigogenesis *in vitro*. To understand the molecular mechanisms coordinated by *Lbr*PRMT5 that are implicated in amastigogenesis, we performed a RNAseq analysis of the META forms of PRMT5-KO in comparison to its parental line (pT007), as well as to a PRMT5-addback line. PCA analysis showed that the alterations in the transcriptome of PRMT5-KO cells were largely restored in PRMT5-addback cells. Importantly, only six genes were found downregulated in PRMT5-KO META forms, while 88 were found upregulated. GO terms related to ncRNA and to macromolecule metabolism were enriched among the upregulated genes, but no enrichment was observed for the downregulated sequences. Using a CRISPR/Cas9 approach, we attempted to knockout all the downregulated genes to assess whether they would be determinant for the META-AMA transition. At this stage, one gene was excluded from the analysis for showing characteristics of transposable elements, and two genes appeared essential for *L. braziliensis* growth and could not be knocked out. The remaining three genes were knocked out: the KO of two (LBRM2903_160008200 and LBRM2903_160019400) reduced the rate of *in vitro* amastigogenesis by ~30%. The double KO of these two genes did not seem to reduce axenic amastigogenesis further. The ability of the KO cell lines to infect macrophages *in vitro* is being assessed. **Supported by:**Fundação de Amparo à Pesquisa do Estado de São Paulo **Keywords:**Leishmania braziliensis;Arginine Methyltransferase;PRMT5.

PV-54 - **Does ATM play a role in DNA repair and genome plasticity in *Leishmania*?**

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To ensure the genome is faithfully transmitted to the next generation, DNA injuries or lesions must be resolved. Failing to repair this damage can promote chromosome breaks, genome instability, tumorigenesis, and cell death. Double Strand Breaks (DSBs) are highly deleterious lesions where both DNA strands are compromised. In eukaryotes, specialised pathways resolve DSBs as part of the wider Damage Response (DDR). Ataxia-telangiectasia Mutated (ATM), is crucial kinase in coordinating DSB repair that is recruited to and activated at DSB sites by a complex called MRN (Mre11-Rad50-Nbs1). Once ATM activates, it triggers a phosphorylation cascade stimulating the recruitment of additional repair factors and promoting accurate DSB repair. In *Leishmania*, a pathogenic protozoan that causes leishmaniasis, recent studies suggest ATM resolves oxidative stress and may play a role in parasite differentiation, but more work is needed to examine the scope of ATM's functionality, particularly during DSB repair in these parasites. We used CRISPR/Cas9 to delete ATM in *L. major* promastigotes, finding that ATM, *in vitro*, is non-essential but loss of the kinase affects parasite proliferation, particular under genotoxic stress conditions, leading to the activation of a previously undescribed damage induced G1/S phase checkpoint. Loss of ATM also coincided with the accumulation of RPA1 foci and enhanced DSB formation, supporting a role for ATM in maintaining genome stability. Given extrachromosomal amplification in these parasites is associated with genome instability, we tested whether ATM may play a role in this process. Indeed, we found that loss of ATM leads to a loss of circular DNA amplification, with future work aimed at resolving this parasite-specific function. **Supported by:**FAPESP **Keywords:**ATM kinase;Double strand breaks;genome instability.

PV-55 - **Unveiling a novel Isoprenoid Salvage Pathway in *Plasmodium falciparum*: new perspectives in fosmidomycin as an antimalarial**

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Malaria, predominantly caused by the *Plasmodium falciparum* pathogen, remains an endemic problem in tropical and subtropical regions. The emergence of drug resistance is a major problem, highlighting the need for new antimalarial compounds. Fosmidomycin (FOS) inhibits the methylerythritol 4-phosphate (MEP) pathway, crucial for isoprene unit biosynthesis. Despite its efficacy, recurrent cases of recrudescence have led to investigations into the biological causes of FOS recovery. This study found isoprenoid alcohols such as farnesol (FOH), geranylgeraniol (GGOH), phytol (POH), and unsaponifiable lipid extracts from foods can restore FOS activity in *P. falciparum*. These substances are phosphorylated, lengthened, and incorporated into proteins. Proteomic and radiolabeling studies showed that prenylated proteins can bind to several isoprenoids if externally supplied. A gene (Pfpolk) encoding a prenol kinase was identified, which when expressed in yeast, exhibited farnesol and geranylgeraniol kinase activities. Conditional knockout parasites (ΔPfpolk) were created using CRISPR-Cas9 and DiCre strategies, to investigate the biological importance of the farnesol/geranylgeraniol salvage pathway. ΔPfpolk parasites were more susceptible to MEP inhibitors and incapable of using isoprenoid alcohols for protein prenylation. The study suggests that the farnesol and geranylgeraniol salvage pathway is an additional isoprenoid source for malaria parasites. Inhibition of this pathway could enhance the effectiveness of drugs targeting isoprenoid metabolism. A compound reducing the FOS-recovering effect of geranylgeraniol was identified, making it a potential candidate for co-use with FOS in trials. Collectively, these findings deepen our understanding of the action mechanisms of antimalarials targeting the apicoplast and shed light on a novel post-translational modification of proteins in *P. falciparum*, providing valuable insights for the development of more effective antimalarial drugs. **Supported by:**FAPESP 2018/02924-9 and 2020/14897-6 **Keywords:**Malaria ;fosmidomycin;isoprenoid.

PV-56 - **U18666A: A tool for understanding intracellular cholesterol trafficking on *Leishmania amazonensis***

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Leishmaniases are part of the neglected tropical diseases caused by different species of protozoa of the genus *Leishmania*. Sterols are structural lipids and are present in the membranes of most eukaryotic cells. Unlike animals, which synthesize cholesterol, *Leishmania* spp. synthesize ergosterol and other ergostane-based sterols. In addition, this parasite can obtain exogenous cholesterol derived from the culture medium or the host cell. Therefore, studying cholesterol uptake by *Leishmania* spp. is fundamental for understanding the role of this sterol in the disease. To develop this study, an amphipathic cationic amine known as U18666A [3β-(2-diethylaminoethoxy)androst-5-en-17-one] was selected. This chemical compound can alter intracellular membrane protein traffic by impairing intracellular biosynthesis and transport of LDL-derived cholesterol. Because of this, it is widely used to address questions about the control of cholesterol biosynthesis, intracellular cholesterol traffic, and traffic of other molecules through endosomal and lysosomal compartments, and in understanding the pathophysiology of various diseases, including epilepsy, cataract, Niemann-Pick type C disease, atherosclerosis, Alzheimer's disease, prion infections, and others. In this work, the efficacy in *Leishmania amazonensis* promastigotes was evaluated. The IC50 in promastigotes of *L. amazonensis* were 0.63 ± 1.0. Cytotoxicity assays of the compound U18666A on macrophage cultures revealed that the compound alone is not toxic to uninfected peritoneal macrophages at the concentrations tested. Trials on amastigotes have shown promising results. This work indicates that U18666A has antileishmanial activity and further studies will evaluate its activity on the sterol composition of the parasite and the effect in combination with azoles. **Supported by:**Faperj E26-201.158/2022 e Faperj E26-210.157/2018 **Keywords:**Leishmania amazonensis ;U18666A;Sterols.

PV-57 - **Insights on The Role of the PAF and LPC Receptor in Cell Biology and Mitochondrial Physiology of Trypanosoma cruzi**

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Phospholipids, such as platelet-activating factor (PAF) and lysophosphatidylcholine (LPC), mediate proliferation, cell differentiation, and infectivity in diverse eukaryotes, including trypanosomatids. Our group identified a gene encoding a putative PAF and LPC receptor in *Trypanosoma cruzi* (TcPAQR) and produced single knockout (SKO) and total knockout (KO) mutants for this gene. Previous ultrastructural analysis revealed alterations in the morphology and mitochondrial ultrastructure of KO parasites when compared to WT cells. Thus, the objective of the present study was to evaluate the importance of this receptor in cell biology and mitochondrial physiology in *T. cruzi*. Comparing the growth curves over 7 days by direct counting, the SKO and KO parasites were found to have a significantly lower proliferative capacity from the 5th day onward. The cell viability of the three phenotypes (WT, SKO, KO) was compared, and the results indicate that all protozoa were viable throughout the experiments. The apoptosis rate of these parasites was also evaluated by the TUNEL method and flow cytometry. The results showed a higher rate of apoptosis for KO compared to WT and SKO parasites. To assess the oxygen consumption of the three phenotypes (WT, SKO, KO), an analysis was performed by respirometry, using modulators of mitochondrial processes, showing that there were no significant differences between the three phenotypes. This indicates that, despite possible damage to mitochondria, the respiratory chain is likely to be functional. However, KO mutants showed a reduction in mitochondrial membrane potential and a high ROS content, which suggests a mechanism yet to be studied to sustain oxygen consumption at normal levels and thus remain alive. This work highlights the importance of the receptor for PAF and LPC in the basic survival mechanisms of *T. cruzi*, which can be explored as a potential target for experimental chemotherapy against Chagas disease. **Supported by:**CNPq, CAPES, FAPERJ, INCTEM **Keywords:**Mitochondria;PAF and LPC receptor;Mutants.

PV-58 - **In silico Characterization of TcPAQR: A Putative Receptor for PAF and LPC in *Trypanosoma cruzi***

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TcPAQR is a homologous protein to members of the progestin and adiponectin family of receptors (PAQRs) in humans. TcPAQR is a transmembrane protein and is considered a putative receptor for the lipid mediators platelet-activating factor (PAF) and lysophosphatidylcholine (LPC), which play a role in *Trypanosoma cruzi* cell differentiation and the infection of peritoneal mouse macrophages. In vitro assays have shown that knockout parasites lacking TcPAQR4 were unable to respond to the stimuli of PAF and LPC in cell differentiation. The main goal of this project was to discover possible drugs to render the infection nonviable in humans. TcPAQR was modeled using AlphaFold, an Artificial Intelligence (AI) program developed by Google DeepMind, known for its accurate protein molecular modeling, especially for proteins with low similarity to homologous structures (<30%). Additionally, modeling was performed using RaptorX, I-Tasser, Modeller, and Phyre2, which are programs based on the structure of known homologous proteins. Modeller required a pdb file for modeling, and the 6KS1 sequence was utilized. For AlphaFold, ColabFold was employed, applying 6 recycling steps, the Amber ForceField, and the pdb70 database. All other settings were left as default. All models were compared using RMSD analysis in PyMOL, and a structure validation analysis was conducted using Saves (UCLA-DOE), which employed VERIFY 3D, ERRAT, PROVE, WHATCHECK, and PROCHECK. Based on the analysis, the AlphaFold model was deemed the closest approximation to reality. Molecular docking of this receptor using Vina will be performed in the near future. **Supported by:**CNPq, CAPES, FAPERJ, INCTEM **Keywords:**PAF and LPC receptor;Molecular modeling;Artificial intelligence.

PV-59 - **One Health Approach to Tackle Cryptosporidiosis and Giardiasis in the Pampa Region of Argentina**

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Cryptosporidiosis and giardiasis are emerging enteritis diseases with zoonotic risks globally. *Giardia and Cryptosporidium primarily impact young individuals, leading to malnutrition and significant economic losses in livestock.* In this work, we aimed to assess *Cryptosporidium* and *Giardia* presence and to identify circulating subgenotypes and risk-associated factors in Córdoba, Argentina. Our study evaluated the copro-parasitological status of 150 asymptomatic children under 15. We collected 374 soil samples, and 493 dog fecal samples, and processed fecal samples from 140 calves under 60 days of age from two dairy farms in the study area. We also assessed nutrition, demographics, and anthropometric data using questionnaires and census information. Dog variables included characteristics, health status, environment, anthelmintic use, veterinary management, and owners' commitment. Data were analyzed using multi-level models, AIC comparison, and generalized linear mixed-effects models in R. Geographic information systems helped create predictive maps for parasitic exposure, malnutrition, animal infections, and combined conditions. In children, five genera of parasites were detected, with *G. duodenalis* being the most common pathogen. Individual and household factors influenced the presence of *Cryptosporidium* and *Giardia*, while neighborhood-level environmental factors also played a role in co-infection. Calves predominantly showed *G. duodenalis* genotype E, and several subgenotypes of *C. parvum* were identified, including a novel subgenotype. The most frequent subgenotype in calves with diarrhea was IIaA20G1R1, followed by IIaA18G1R1. These subgenotypes were also found in humans, indicating a potential for zoonotic transmission. The study's findings are significant for understanding the zoonotic potential of gastrointestinal protozoan parasites and their impact on public health in the Córdoba province, highlighting the importance of One Health approaches. **Supported by:**Agencia Nacional para la Promoción de la Ciencia y Tecnología, Argentina, grant number PICT2018-713 and PICT-2021-CAT-II-00073.
Keywords:0NE HELTH;parasitic infections;zoonosis.

PV-60 - **Investigating Growth Patterns and Species Interactions of Trypanosomatid Parasites in a Cultured Environment**

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In this study, individual growth curves were generated for five different parasites, namely *Strigomonas oncopelti*, *Crithidia fasciculata, Leishmania amazonensis*, *Phytomonas serpens*, and *Trypanosoma cruzi*. The parasites were cultured in liver infusion tryptose (LIT) medium supplemented with 0.002% hemin and 10% heat-inactivated fetal calf serum at 28°C, pH 7.2. An inoculum of $5x10^5$/ml in 5 ml was used. Subsequently, all five parasites were combined and added to the same culture medium ($5x10^5$/ml in 5 ml). The parasite pool was harvested daily, and the culture medium was replaced with fresh medium. Each species of trypanosomatids was counted daily for a period of 14 days using a Neubauer chamber. The resulting percentages of each parasite were plotted. To further investigate this phenomenon, a similar experiment was conducted using only three species (*S. oncopelti*, *P. serpens*, and *T. cruzi*), and the same pattern emerged. *T. cruzi* disappeared from the culture around day 6-7, while *P. serpens* was found in very low numbers from day 11 onwards. *S. oncopelti*, on the other hand, persisted in high numbers until the 14^{th} day of culture. Each experiment was replicated five times in triplicate. Currently, ongoing experiments are being conducted to shed light on the mechanisms underlying this phenomenon. **Supported by:**CNPq, CAPES, FAPERJ, INCTEM **Keywords:**Strigomonas oncopelti;Phytomonas serpens;Trypanosoma cruzi.

PV-61 - **Biochemical characterization of cytokinin biosynthesis in *Plasmodiu ciparum***

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Malaria is a parasitic infectious disease caused by P th problem and a risk to diverse populations. Pre ase poses a threat to 40% of the world&#39 primarily observed in isolates of *Plasmodiu* drugs. In this regard, the biosynthesis of iso gained prominence as a potential target for th s disease. This is due to the fact that the co survival of any cell, and the biosynthetic p iosynthesis pathway in humans. Isopreno nesis regulation in plants. Therefore, we aim in the intraerythrocytic forms of *Plasmodium* egulating the parasite's cell cycle. Our results nesis in *Plasmodium falciparum*, and we observed the e duced cytokinin biosynthesis and viability in P. falciparum, as we ization. The compound forchlorfenuron decreased parasitemia and velopmental stages of the P. falciparum cycle. Understanding the bioch in cytokinin biosynthesis will contribute to a better understanding of cytokinin functio *sp.*, and guide the development of new therapies for the treatment of malaria with *Plasmodium falciparum*. **Supported by:**CAPES processo: 88887.473720/2020-00 (PROE , **Keywords:**Malaria;phytohormones;cytokinins.

PV-62 - **Prospection of C24-methyltransferase (ERG6) inhibitors for the treatment of leishmaniasis**

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In searching for the rational development of new drugs, choosing a selective target in the parasite is essential. The divergent step of the parasite lipid biosynthesis pathway is the transfer of one methyl to carbon 24 of sterols with a cholestane structure, forming a branch in C24 not existing in mammalian sterols catalyzed by the C24-sterol methyltransferase enzyme (ERG6). This work aims to evaluate azasterols with different modifications in their structure as inhibitors of the enzyme ERG6 of *Leishmania* spp. For this purpose, we used strains of *Leishmania infantum* and *Leishmania amazonensis* that overexpress ERG6 to perform a drug screening. The azasterols evaluated in this work were synthesized by our collaborators. Finasteride and dutasteride were acquired from Sigma-Aldrich and also had their leishmanicidal activity evaluated for belonging to the class of azasteroids. Confirmation of increased in gene transcripts in both *Leishmania* strains was confirmed by quantitative PCR. In the transfected promastigotes of *L. amazonensis* (Laerg6^{high}), it was possible to observe an increase of the relative expression of mRNA of ERG6 of approximately 17 times compared to the wild strain, while in *L. infantum* strain (Lierg6^{high}) we observed an increase of approximately 45 times. Then, promastigotes Lierg6^{high}, Laerg6^{high}, *L. amazonensis* WT and *L. infantum* WT were incubated with test molecules at different concentrations and with dutasteride and finasteride for 72 hours. Afterward, the growth of parasites was evaluated with resazurin. All azasterol derivatives of the ND series showed leishmanicidal activity against promastigotes of *Leishmania* spp. Additional tests will be performed with the Lierg6^{high} and Laerg6^{high} strains, including antiamastigote activity, toxicity, evaluation of the sterol profile, and molecular docking. **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:**Azasterols;Leishmania;24-C-methyltransferase.

PV-63 - **Extracellular vesicles from different virulence profiles Leishmania amazonensis plays a important role in parasite-parasite communication.**

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Leishmaniasis is a group of infectious diseases caused by *Leishmania spp*. It is estimated that up to 1 million new leishmaniasis cases occur every year, with 65,000 deaths occurring annually. Components expressed or secreted by *Leishmania sp.*, identified as virulence factors (VF), can participate in host's immune response subversion, playing a key role in infection success. *Leishmania spp.* can spontaneously release extracellular vesicles (EVs) containing parasite's antigens, VFs, RNA, DNA, and lipids. Data shows that EVs play a key role in the parasite-host relationship due to their ability to carry antigens and modulate the immune response. Studies shown that EVs can be a potential drug target for leishmaniasis treatment, as they also act by modulating the immune response, favoring the infection. Therefore, identifying its role in inter-parasite communication is of great interest. Thus, our objective was to verify VF genes expression in *In vivo*-derived (IVD-P (Virulent profile)) and Long-term *In vitro* culture (LT-P (Attenuated profile)) *L. amazonensis*, and perform EV transfer assays. For this purpose, both virulence profiles *L. amazonensis* were cultivated and RNAs were extracted for VF gene expression analysis using q-PCR. After, IVD-P and LT-P were cultured and EVs purified for transfer assays. EVs were stained with PKH-26 and promastigotes were analyzed by flow cytometry. Our results showed that IVD-P parasites had significantly increased LPG3 and GP63 genes expression when compared to LT-P. Transfer assays showed that IVD-P and LT-P parasites were able to internalize EVs originating from both IVD-P and LT-P strain. Also, confocal microscopy acquired images identified and certified the interaction between promastigotes and both IVD-P and LT-P EVs. In conclusion, *L. amazonenis* can modulate virulence genes expression depending on stimuli. Also, our study showed, in an unprecedented way, that *L. amazonensis* can communicate with adjacent parasites via EVs. **Supported by:**CNPq, CAPES, FAPESP **Keywords:**Leishmania amazonensis;Extracelular Vesicles;Transfer assay.

PV-64 - **Investigating the role of inositol pyrophosphates in cell survival, virulence, and maintenance of genomic integrity of *Leishmania braziliensis***

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Leishmaniases are a group of diseases caused by parasites of the genus *Leishmania* (Trypanosomatidae family). Currently, there are no vaccines or prophylactic measures, and existing drugs are toxic to the patients. In model eukaryotes, inositol pyrophosphates (PP-IPs), mainly IP_7 and IP_8 are involved in a wide range of cellular processes, such as regulation of telomere length and in the maintenance of virulence in some pathogenic fungi. The participation of IP6K and PP-IP5K kinases are essential in the IP_7 and IP_8 biosynthesis pathway, respectively. However, trypanosomatid parasites do not have orthologous genes for PP-IP5K, which makes them excellent models for studying the role of IP6K and consequently IP_7. In this study, lineages of *L. braziliensis* KO for IP6K ($IP6K^{-/+}$), telomerase ($TERT^{-/-}$) and double-KO ($IP6K^{-/+}$ + $TERT^{-/-}$) were generated using CRISPR/Cas9 approach to investigate the role of IP_7 in telomere homeostasis and its contribution to the virulence of this protozoan. The RT-PCR and RT-qPCR assays revealed the successful application of the CRISPR/Cas9 system in *L. braziliensis*. Although immunofluorescence assays (IFA) using α-γH2A revealed an accumulation of DNA damage in double-KO lineage ($IP6K^{-/+}$ + $TERT^{-/-}$), growth curves did not show a significant decline in their proliferation relative to the WT lineage. Moreover, DNA content analyses using flow cytometry did not indicate any cell cycle arrest. Currently, we are investigating where, in the genome, the DNA damage detected is occurring. Also, we are investigating possible morphological alterations due to the removal of IP6K. Subsequently, the lineages generated will be challenged with some genotoxic drugs to shorten telomeres (e.g.: H_2O_2), which will contribute to the understanding of the role of PP-IPs during telomere elongation in trypanosomatids. Of note, this work aims to shed light on the search for new potential molecular targets that can be used for the development new drugs against leishmaniases **Supported by:**FAPESP: 2022/00923-0
Keywords: Inositol Pyrophosphates;CRISPR/Cas9;Leishmania braziliensis.

PV-65 - **Pseudopod Projection and Modulation: Exploring Variability Across the Tree of Amoebozoan Testate Amoebae (Arcellinida: Amoebozoa)**

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Testate amoebae are unicellular eukaryotes provided with an outer covering (shell). These organisms protrude pseudopods through the single shell aperture and use them for locomotion, feeding, and other complex behaviors. The pseudopod is considered a key morphological character, and it is used to describe, distinguish, and classify amoeba groups. Despite the central role of pseudopods, the intra-specific morphological variability of this structure and the relationship between this variability and other features of the organisms has been poorly explored, including the correlation between this variability and different substrate types. In this study, we sampled representative species from two diverse infraorders of Arcellinida (Amoebozoa) that inhabit different natural environments and substrates. Using light microscopy, we observed their behavior on three different substrate types: agar plates, plastic bottles, and glass plates with liquid media. The primary objectives were to investigate the range of morphological variability in relation to organismal performance (i.e., locomotion speed), examine whether shell attributes and substrate types correlate with performance, and describe the nature of these correlations and their interplay with the variables. To achieve these objectives, we measured various features including shell diameter and area, pseudopod number per individual, and pseudopod length and width of multiple individuals. We then used these measurements and locomotion speed as variables in generalized linear models (GLMs) for further analysis, constructing separate models for each substrate type. The present study sheds ligth on the variability of pseudopod projection and modulation across the tree of amoebozoan testate amoebae (Arcellinida). Investigating pseudopod variability within Arcellinida leads to a better understanding of their diversity and differential capability to inhabit diverse habitats. **Supported by:**CAPES **Keywords:**cellular motility;microeukaryotic plasticity;variability.

PV-66 - **Consequences of IP6K disruption for Trypanosoma cruzi: the conundrum of inositol pyrophosphates pathway**

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Chagas disease is a neglected tropical disease caused by the protozoan parasite *Trypanosoma cruzi*. There are no effective vaccines and the available treatment options are effective only in the acute phase of the disease but can cause serious side effects on the patients. Thus, the search for the elucidation of molecular pathways that may provide potential targets for drug development is of paramount importance. Inositol pyrophosphates (PP-IPs) – mainly IP_7, and IP_8 – are involved in a wide range of processes in eukaryotes. However, the mechanism of action of these metabolites is not yet fully understood. IP_7 and IP_8 are synthesized by pathways involving the participation of IP6K and PP-IP5K kinases, respectively. Trypanosomatids have an ortholog gene for IP6K but do not have orthologs for PP-IP5K, making them excellent models for studying IP_7. Here, using CRISPR/Cas9 approach, we disrupted single and double alleles of IP6K, generating $IP6K^{-/+}$ and $IP6K^{-/-}$ lineages, respectively. IP6K inactivation causes several morphological effects, such as rounding and wrinkling of the cell body, increased number of glycosomes, and mitochondrial enlargement. Notably, $IP6K^{-/-}$ lineage (double-null) was unable to proliferate for more than 7 days, suggesting that this kinase is essential for this organism. Interestingly, $IP6K^{-/+}$ lineage (single-null) showed a slight cell cycle arrest at G0/G1 phase with no DNA damage (quiescent cells). Moreover, the $IP6K^{-/+}$ lineage showed a high proliferative capacity within the host mammalian cell, suggesting that the quiescent cells observed may be metacyclic forms. Together, our findings suggest that the total loss of IP6K has harmful consequences for *T. cruzi*. However, paradoxically, the disruption of a single allele of IP6K leads to *T. cruzi* being prone to multiplying further inside the host cell, generating a conundrum that we are still trying to understand. **Supported by:**FAPESP: 2020/16480-5
Keywords:CRISPR/Cas9;Inositol Pyrophosphates;Quiescence.

PV-67 - **Endosymbiosis in trypanosomatids: a study of the cell cycle in species of the *Kentomonas* genus**

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Kentomonas is a new genus of monoxenic trypanosomatid containing an endosymbiotic β-Proteobacterium that presents phylogenetic proximity to the symbiotic preparations present in *Angomonas* and *Strigomonas* genera. The relationship between prokaryotes and protozoan is mutualistic and characterized by intense metabolic exchanges, lost changes in associated partners, and bacterial division synchrony with other host cell structures. Considering these aspects, symbiosis in trypanosomatids represents an interesting model for evolutionary studies and also for comparison with heteroxenic trypanosomatids that are pathogenic to humans. This work aims to characterize the cell cycle of *K. deaneorum* and *K. sorsogonicus* and compare it with *A. deanei* and *S. culicis*. For that, we used growth curves, transmission electron microscopy (TEM), fluorescence microscopy (FM) and flow cytometry. Our results showed that the generation time of *K. deaneorum* is 4h, while that of *K. sorsogonicus* is 6h. TEM revealed that in the first species, the symbiont is closely associated with mitochondria, while in the second there is proximity between bacteria and glycosomes. An anti-porin antibody and DAPI revealed that the first structure to replicate is a symbiotic bacterium, which seems to use the nucleus as a topological reference during its division, followed by the kinetoplast and then the nucleus, as observed in *A. deanei* and *S. culicis*. We analyzed the cell cycle of both species by flow cytometry and established a protocol for synchronization using hydroxyurea, which caused an arrest in the G1 phase. In both species of *Kentomonas*, there is an adjustment of symbiont division along the host cell cycle, so that each generated cell has a single bacterium and a single copy of the nucleus and kinetoplast. Taken together, our results propose that trypanosomatids harboring symbionts represent an excellent model for studying the origin of organelles in the eukaryotic cell. Supported by FAPERJ and CNPq **Supported by:**161032/2021-4
Keywords:cell cycle;trypanosomatids;symbiosis.

PV-68 - **Optimization of Ultrastructural Expansion Microscopy (U-ExM) to study cytoskeletal structures and different organelles in *Trypanosoma cruzi***

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Ultrastructural Expansion Microscopy (U-ExM), is a method to improve the resolution of optical microscopes through the physical expansion of a sample. During U-ExM, a swellable polymer network (or hydrogel) is synthesized uniformly throughout a biological sample or tissue. The samples then undergo a process of mechanical softening and homogenization, followed by a process of isotropic three-dimensional physical expansion when water is added, causing the polymer and thus the sample in which the polymer is embedded in to enlarge. As a result, the biomolecules of interest become spatially separated from each other and the effective resolution of the microscope increases. This technique is compatible with standard immunofluorescence protocols.
We optimized an U-ExM protocol for *Trypanosoma cruzi* and quantifying the expansion factors of different subcellular compartments and organelles. We determine the localization patterns of different tubulin isoforms, such as α-tubulin and β-tubulin. Also we immunolocalized acetylated and tyrosinated α-tubulin isotypes in epimastigotes and use mitochondrial cell-permeable dyes to identify this organelle. Finally, U-ExM was also performed in trypomastigotes and amastigotes validating this technique in all life cycle stages of *T. cruzi*. We observed that the typical morphology of the epimastigotes is preserved during the process, achieving an expansion factor of 4.5 times. We analyzed the expansion of the nucleus, kinetoplast, and basal body to confirm the expansion by measuring their size with ImageJ software. These results allow us to corroborate the usefulness of this technique for *T. cruzi*, especially for the analysis of cytoskeletal proteins. Also, it is possible to appreciate within the nucleus areas with different fluorescence intensity, which allows distinguishing areas with different chromatin compaction without having to use electron microscopy or other super-resolution techniques. **Supported by:**Agencia Nacional de Promoción Cientifica y Tecnológica, Argentina (PICT2019-0526, PICT2021-0157), CONICET (PIBAA 1242)
Keywords: Expansion Microscopy;Cytoskeleton;Mitochondria.

PV-69 - **Single copy gene tagging as an alternative approach to study chromosomal aneuploidy in *Trypanosoma cruzi* by FISH**

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Recent studies have demonstrated considerable genomic variability and complex patterns of aneuploidy in *Trypanosoma cruzi*. However, the extent of variation and the classification of these aneuploidies into segmental or whole chromosome aneuploidy is still unknown. Here we investigated by Fluorescence In Situ Hybridization (FISH) the presence of aneuploidy in chromosome 24 (TcChr24) of *T. cruzi* (clone CL Brener) which presents evidence of trisomy by NGS. Initially, we analyzed the chromosomal ploidy of TcChr24 using as chromosome-specific markers three single copy genes mapped in this chromosome. It was possible to quantify the aneuploidy events in the populations from markers (TcCLB.503515.20, TcCLB.506413.70). However, It was not possible to estimate aneuploidy using the TcCLB.509127.80 as marker because it did not show sufficient labeling efficiency for quantification. Our data show that, for both genes, there is a higher percentage of monosomic cells, followed by disomic, trisomic and, rarely, polysomic cells. Then, we developed a new tool to analyze the aneuploidy events of the TcChr24, considering all three markers. We generated mutant parasites by CRISPR/Cas9, inserting an exogenous tag, in these three single copy genes, aiming to better understand the nature of the aneuploidy, whether whole or segmental chromosomal. Using the tag sequence as a probe, we achieved labeling efficiency above 85% for the three genes. Also, our data showed that most cells present monosomy, followed by disomy, trisomy, and polysomy for the three markers. This data corroborates what we found before in untagged cells, suggesting the occurrence of chromosomal somy variation for TcChr24. In conclusion, our data demonstrate the occurrence of non-homogeneously distributed aneuploidies in the population, that is, the subpopulations display cell-to-cell somy variation for a given chromosome, suggesting the occurrence of mosaic aneuploidy. **Supported by:**FAPESP (PD 2019/05049-4 and Thematic Project 2016/15000-4) and CNPq **Keywords:**CRISPR/Cas9;Aneuploidy;FISH.

PV-70 - **Exploring the Epidemiology, Genotypes, and Genetic Variability of *Toxoplasma gondii* in Sheep Populations of Uruguay**

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Sheep husbandry has been central to the economic and social development of Uruguay throughout history. Uruguay is currently the fifth largest exporter of sheep meat and third of combed wool in the world. However, reproductive indexes for the species is far from ideal. Our previous studies determined a high incidence of embryonic and fetal losses due to infection by *Toxoplasma gondii* whereby 58% of abortions of infectious etiology are due to this parasite). However, the prevalence of the parasite at the population level and the prevalent genotypes present in sheep flocks in Uruguay are unknown. Here, we determine the prevalent genotypes causing abortion in sheep by molecular and serological techniques. In addition, we isolated two novel strains from sheep (TgUru1 and TgUru2) and characterized them both genetically and phenotypically both *in vivo* and *in vitro*. Our findings suggest a broad genetic variability in the country, with atypical and unreported genetic types being predominant. Moreover, TgUru1 and 2 display radically distinct phenotypes, whereby the former is highly virulent and fast growing, whilst the latter is slow growing and displays high rates of spontaneous cystogenesis. We are currently analyzing the genetic and transcriptomic bases of these differences and their potential impact on the disease outcome. Additionally, we are exploring the correlation between our animal findings and the human population in the country in terms of the prevalent strain's genetic background. **Supported by:**Fondo Sectorial Salud Animal en ANII
Keywords: Sheep husbandry;Toxoplasma gondii;genotypes.

PV-71 - **Deciphering the role of YEATS domains in *Leishmania mexicana***

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Post-translational modifications (PTMs) govern crucial regulatory mechanisms in diverse organisms, influencing various biological processes. In 2011, lysine crotonylation, a novel PTM, was identified in histones. The crotonyl group, characterized by a rigid and flat C-C structure, has been found to modulate gene transcription and other vital cellular processes when added to the N-terminal chains of histones. The intricate regulation of crotonylation levels relies on crotonyltransferases, decrotonylases, and the readers, known as YEATS proteins. YEATS domains facilitate the binding of crotonylated lysines, facilitating the recruitment of regulatory proteins and triggering downstream effects. This study focuses on the characterization of YEATS domains in the protozoan parasite *Leishmania mexicana*. Utilizing global alignment analysis based on known YEATS domains from other organisms, we identified two YEATS-domain-containing proteins in *L. mexicana*, namely ENL and Yaf9. Protein structure modeling revealed conserved amino acids within the ß-strand 'sandwich' region, important for crotonyl group interaction. Furthermore, we observed distinct expression patterns of ENL and Yaf9 among parasite stages, suggesting their involvement in stage differentiation processes. To gain deeper insights into the biological functions of YEATS proteins in *L. mexicana*, we generated parasite overexpressing ENL (ENL-ox) and investigated their subcellular localization in the procyclic stage. Remarkably, we observed a stage-dependent differential nuclear localization pattern of ENL, with perinuclear distribution during non-dividing stages and dispersed nuclear localization during dividing stages. Moreover, a slight decrease in procyclic stage multiplication was observed in ENL-ox parasites compared to the wild-type cells. Future investigations will involve additional phenotypic analyses to comprehensively elucidate the role of crotonylation in *Leishmania* biology. **Supported by:**FAPESP 2021/04748-6
Keywords: Leishmania;Crotonylation;YEATS.

PV-72 - **Comparative molecular analysis of *Lutzomyia umbratilis*, the main vector of *Leishmania (viannia) guyanensis*, from endemic and non-endemic leishmaniasis regions of the Amazon state.**

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The epidemiological profile of the American Cutaneous Leishmaniasis in the Amazonian cities of Manacapuru (MNP) and Rio Preto da Eva (RPE), separated by the Negro River, differs, RPE being an endemic area of the disease and MNP showing no significant number of cases. With the aim of identifying molecular difference between the two populations, we performed midgut proteomic analysis . A total of 3292 proteins were identified, 3252 of which are shared by sandflies from both locations, 30 are exclusively present in MNP and 11 only in RPE. Among the proteins, eight were selected for transcriptional validation, based on the criteria of amount of reads and having a potential to impact on the vectorial capacity of insects. Of these eight proteins, four were only detected in MNP: SARA (Smad anchor for receptor activation), Citocromo P450, CysPc (Calcium-dependent cytoplasmic cysteine proteinases) and XPR1 (Xenotropic and Polytropic Retrovirus Receptor 1). The other four proteins were detected both in MNP and RPE, but they had a fold change greater than 2 or less than 0.5 in MNP: SL9B2 (Sodium/hydrogen exchanger 9B2), PATH (Proton Coupled Amino Acid Transporter Like Protein Pathetic), PEPT1-Like (Peptide Transporter 1-Like) and Paramiosin. With these targets defined, RT-qPCR was performed using insects from the two locations to compare the level of transcripts in carcasses and intestinal tracts. Our results showed that most of these proteins are upregulated in MNP, similar to the data from the proteomic analysis and are probably relevant in the mechanism of parasite transmission by the insect. We are now in the process of silencing some selected genes to analize their effect on infection. Using the proteomic data, we identified bacterial proteins and analized the microbiota composition of both populations, The possible implications of these data are being analysed in relation to the different vectorial capacity of these sand fly populations. **Supported by:**FAPERJ, INOVA-FIOCRUZ e IOC-FIOCRUZ
Keywords: Leishmania;Sand fly;Vectorial capacity.

PV-73 - **Effects of nalidixic acid on *Trypanosoma cruzi* evidences associated activity of kinetoplast proteins**

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Trypanosoma cruzi has a heteroxenic life cycle that presents structural variations during the cell differentiation process, which is known as metacyclogenesis. The non-infective epimastigote form is found in the intestine of the insect vector and is characterized by a disc-shaped kinetoplast containing a highly compacted mitochondrial DNA (kDNA). Topoisomerase II (topo II) and KAPs (Kinetoplast Associated Proteins) are essential enzymes to the kDNA replication, that involves the release of minicircles to the network. Nalidixic acid pertains to the quinolone group and is a potent topo II inhibitor, capable of blocking T. cruzi cell proliferation and promoting ultrastructural changes in the kinetoplast. In this work we used different methods, such as growth curves and analyzes by different microscopy techniques to verify if topo II inhibition differentially affects mutant cells of KAP7, a protein that has been related to kDNA repair mechanisms in this protozoan. For this purpose, we used WT DM28c cells, protozoa expressing KAP 7 fused to the fluorescent tag (mNG) and Myc, obtained by CRISPR-Cas9 and also protozoa with double knockout for KAP 7. The growth curves showed an inhibition of proliferation after treatment with the inhibitor in a concentration-dependent manner in the three cell types, especially in KO cells. Images obtained by transmission electron microscopy showed alterations in the kDNA arrangement, with the mutant strain being the most affected. Fluorescence microscopy showed the location of KAP 7 in the antipodal sites, the same place where topoisomerases II has been reported. The obtained data showed that the inhibition of topo II affects more strongly the cells with gene deletion for KAP7, indicating that these two enzymes have associated activities. However, further studies are necessary to determine if there is an interaction between KAP 7 and topoisomerase II. **Supported by:**PIBIC - UFRJ e Faperj **Keywords:**nalidixic acid;Kinetoplast Associated Proteins;Trypanosoma cruzi.

PV-74 - **Characterization of the Angomonas deanei microtubule cytoskeleton reveals new insights into symbiotic bacteria division**

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Some trypanosomatid species co-evolve with a symbiotic bacterium in a mutualistic relationship. Among such protozoa, Angomonas deanei stands out for presenting a better characterized genome, which allowed the development of tools for gene deletion. Previous analyzes of the host trypanosomatid cell cycle, showed a coordinated division between the bacteria and other cellular structures, resulting in the generation of daughter cells with a single symbiont. Studies using the RNAi system to modulate tubulin expression have shown that symbiont division is microtubule dependent. In this work, we used Trichostatin A (TSA), an inhibitor of histone deacetylases (HDACs) and also HDAC6 mutant cells obtained by CRISPR-Cas9 to better characterize the cytoskeleton of A. deanei and to verify whether the division of the symbiont is related to dynamic instability of microtubules. Treatment with TSA caused proliferation inhibition and affected the cell survival of mutant cells. The use of different optical and electron microscopy techniques showed morphological and ultrastructural alterations, both in TSA-treated cells and in mutant protozoa. Images obtained by fluorescence optical microscopy showed the filamentation of the symbiont, while those with negative stainning suggested changes in the distribution of microtubules. Cytometry data indicated cell cycle arrest in the G1 phase in TSA-treated cells, but not in HDAC6 mutant parasites. We conclude that the inhibition of deacetylation modifies structural aspects of the protozoan and is able to affect the division of the symbiont, suggesting that this event depends on the dynamism of the host microtubules.
Supported by:PIBIC-CNPq / 121774/2022-8 **Keywords:**symbiosis;trypanosomatids;division synchrony.

PV-75 - **Preliminary characterization of yeasts as potential symbionts of the mycobiota of the triatomine gut**

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In *Rhodnius prolixus*, a key vector of the Chagas's disease, shotgun metagenomic sequencing has unveiled a diverse range of bacteria involved in B-complex vitamin synthesis. However, the understanding of the fungal microbiota in triatomine insect remains limited. In this work, we aimed to characterize the diversity of the fungal gut microbiota of triatomines in order to identify yeast species known to establish symbiotic relationships with their hosts. We first analyzed the mycobiota associated with the digestive tract of triatomines captured in the field using two different approaches: amplification and sequencing of the D1/D2 26S rDNA from insect gut extracts, or axenic cultures of fungal species followed by morphological characterization and molecular identification of the isolates. A total of 90 fungal isolates were identified in the gut of 518 triatomines. Most of the identified species were classified as entomopathogenic, except for two yeast species of the genus *Aureobasidium* and *Trichosporon*. To investigate the nature of this association and its effects on insect physiology, *R. prolixus* first-stage nymphs were artificially fed with the two yeasts. We observed no impact of yeasts on mortality and insect physiology. Although yeast numbers decreased and became undetectable in insect homogenates by the fourth week after feeding, the presence of 26S rDNA amplicons persisted in the samples for a longer period after yeast infections. This suggests a possible association of the yeast with the insect tissues. We next investigated whether yeasts can use urate as their sole source of carbon and nitrogen, as urate is the end product of blood degradation in triatomine insects, found in high concentrations. We showed that both yeasts possess the ability to degrade urate. These preliminary results should contribute to a better understanding of the mycobiota and its role in the vector that could be used to develop strategies to control pathogen transmission by the vector. **Supported by:**CAPES **Keywords:**Mycobiota;Triatomines;Symbionts.

PV-76 - **Decoding the nuclear proteome of *Trypanosoma cruzi* across the cell cycle: insights into metabolic compartmentalization and epigenetics**

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The life cycle of *Trypanosoma cruzi* involves alternating forms between insect (replicative noninfective) and vertebrate (infective nonreplicative) hosts, posing challenges to parasite survival. This transition is accompanied by metabolic reprogramming from carbohydrates to amino acids as the primary energy source. Differentiation induces morphological alterations in the nucleus, including position, shape, and increased heterochromatin. These morphological changes are possibly linked to the role of histone post-translational modifications (PTMs) in chromatin organization, DNA replication, cell cycle and gene expression. Our main goal was to understand the dynamics of nuclear proteins and histone PTMs during the cell cycle using quantitative proteomic approaches. We synchronized the cell cycle with hydroxyurea, obtaining cells at G1/S, S, and G2/M. We isolated nuclei and histones and analysed the proteomes using LC-MS/MS with TMT labelling for nuclear proteins and untargeted methods for histone PTMs. We identified over 2,500 proteins, with 900 quantified within the nuclear proteome. Gene Ontology analysis was enriched in terms related to the "nucleus", "chromatin", and "chromosomes", supporting nuclear extract. Among the identified proteins,364 exhibited differential abundance throughout the cell cycle, including those involved in DNA and RNA processing and heat shock proteins (HSPs). Our preliminary findings indicated enrichment of proteins in metabolic pathways, such as "pyruvate metabolism," "tricarboxylic acid cycle (TCA)", "glucose/gluconeogenesis", and "L-histidine biosynthesis" at G1/S, suggesting potential metabolic compartmentalization in *T. cruzi* nucleus. The ongoing analysis aims to unravel the cell-cycle dynamics of nuclear metabolic proteins and explore possible correlations with histone PTMs. Understanding these mechanisms, we will provide valuable insights into *T.cruzi* cellular processes, survival strategies, and adaptive capabilities. **Supported by:**Fundação de Amparo a Pesquisa do Estado de São Paulo / **Keywords:**Trypanosoma cruzi;Cell cycle;Proteome.

PV-77 - **Quiescence in *Leishmania*, a target for a new generation of chemotherapeutic innovations**

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Quiescence is a reversible cell division arrest allowing cells to survive environmental insults and driven by a dynamic and regulated cell and metabolic remodeling program. Research on this essential adaptive skill in *Leishmania* is still in its infancy, yet it could explain many (sub-)clinical features of the disease. To characterize molecular mechanisms that control the emergence, and the dynamics of quiescence we developed in vitro models for dermotropic *L. braziliensis/L. lainsoni* and viscerotropic *L. donovani*. We developed and validated a quiescence biomarker (lower expression of GFP in rRNA locus) and observed a quiescent sub-population in cells exposed to stationary starvation, and this in both amastigote and promastigote life stages. In *L. lainsoni*, using bulk 'omic approaches, we found a dramatic decrease in the overall transcripts and low molecular weight metabolites in quiescent cells. However, specific genes and metabolites were relatively upregulated, for example autophagy-related genes, amastins, membrane-localized proteins and several polyhexoses and free fatty acids. In *L. donovani*, we used single cell RNAseq to monitor the modulation of transcriptome during the entire life cycle: in amastigotes, we discovered different sub-populations of quiescent cells, hereby highlighting the heterogeneity of the adaptive phenotype. We identified transcriptional markers of the respective sub-populations and we are currently functionally validating candidate drivers/regulators of quiescence. We also studied quiescence in the context of exposure to trivalent antimonials (PAT). Pre-existing quiescence provides drug tolerance and reciprocally, exposure to PAT can trigger quiescence, with an intensity varying among clinical isolates of a same species. Prospectively, our in vitro models could be used in R&D for untargeted screening of compound libraries, the identified drivers/regulators of quiescence could be targeted by specific inhibitors.
Supported by: FWO, Flemish Fund for Scientific Research **Keywords:** Palavras-chave: Quiescence;Drug tolerance;Single cell transcriptomics.

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.
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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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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.

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