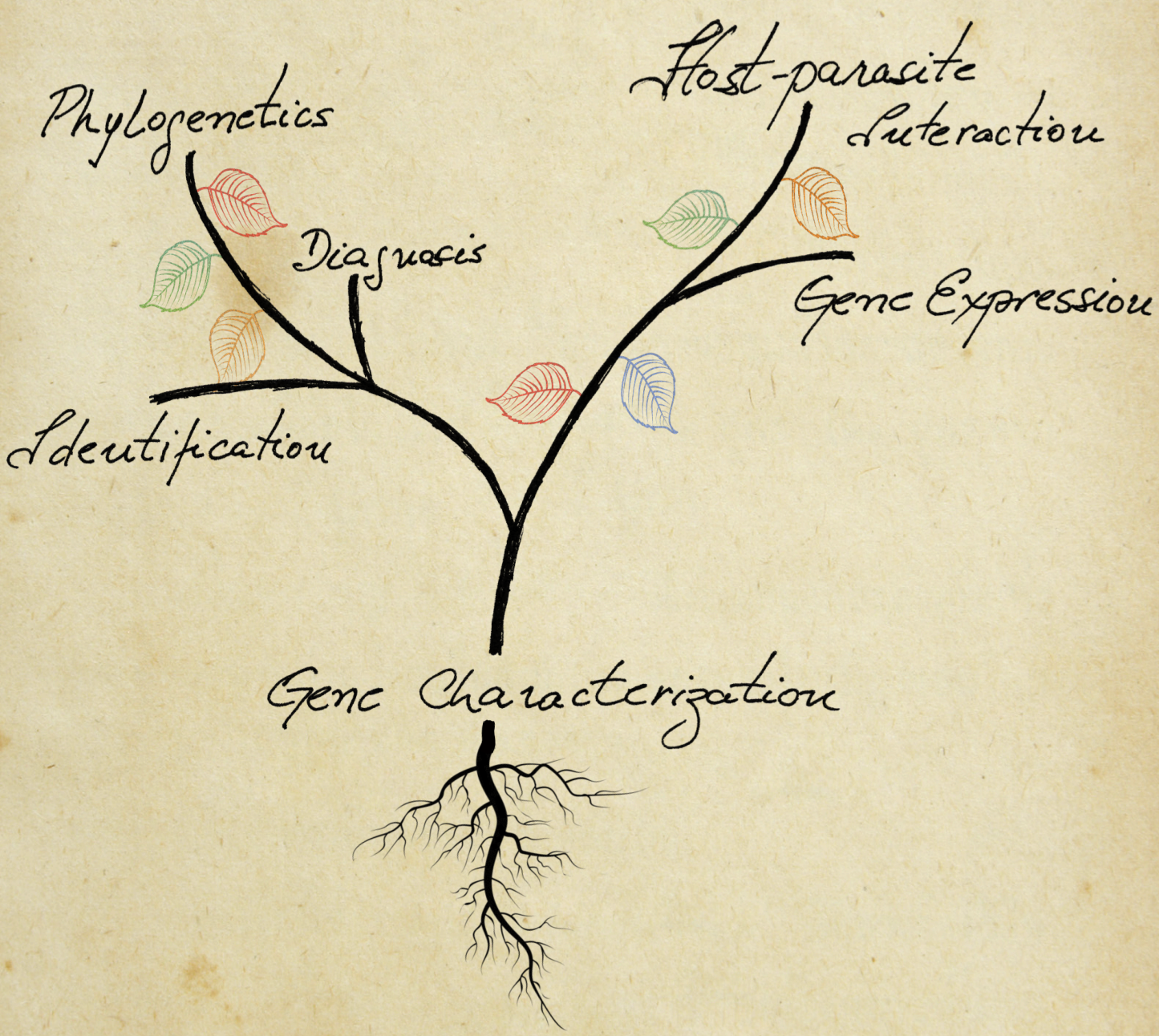
XXXVII Annual Meeting of the Brazilian Society of Protozoology

48th Annual Meeting of Basic Research in Chagas' Disease



Aug 22 - Ago 24, 2022 Hotel Gloria | Caxambu, MG

SBPz

PROCEEDINGS

XXXVII Meeting of the Brazilian Society of Protozoology
XLVIII Annual Meeting on Basic Research in Chagas' Disease

*Hotel Glória, Caxambu, MG, BRASIL- C*axambu
ON LINE
22nd – 24th August, 2022

Colegiado Diretor SBPz

DIRETORIA

Presidente
Luís Carlos Crocco Afonso, UFOP, Ouro Preto, MG, Brasil
afonso@ufop.edu.br

Vice Presidente
Camila Indiani de Oliveira, CPqGM-Fiocruz, Salvador, BA, Brasil
camila.indiani@fiocruz.br

Tesoureiro
Daniel José Galafasse Lahr, USP-IB, São Paulo, SP, Brasil
dlahr@ib.usp.br

Secretária
Renata Rosito Tonelli, UNIFESP, São Paulo, SP, Brasil
r.tonelli@unifesp.br

Presidente anterior
Lucile Maria Floeter-Winter, USP-IB, São Paulo, SP, Brasil
lucile@ib.usp.br

CONSELHO

04/2020 - 03/2024 - Jeffrey Jon Shaw
04/2020 - 03/2024 - Ariel Mariano Siber
04/2020 - 03/2024 - Maria Cristina Machado Motta
04/2020 - 03/2024 - Walter Colli
04/2020 - 03/2026 - Carlos Renato Machado
04/2020 - 03/2026 - Luciana de Oliveira Andrade
04/2020 - 03/2026 - Wanderson Duarte da Rocha
04/2020 - 03/2026 - Yara Maria Traub Cseko

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 **XXXVII Meeting of the Brazilian Society of Protozoology** and the **XLVIII Annual Meeting of Basic Research in Chagas´ Disease**.

After two years we are excited to be back to Caxambu with our meeting. This year the meeting will be held in a hybrid format where some participants will present their talks online.

We would like to acknowledge all the colleagues who submitted their suggestions to the program. Unfortunately, we were not able to include all the excellent suggestions due to space and time limitations. Speakers not included in the present meeting will, obviously be considered for the next years.

As usual we invited speakers from many countries and from different regions of Brazil in an attempt to bring to you the best science in the field of Protozoology. A total of ten conferences and four round tables with four speakers in each will discuss the advances in the area.

In keeping with the meeting tradition four sessions of oral presentations and two poster sessions will allow for students and post-docs to present 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 Lucile Floeter Winter in recognition to her outstanding contribution to the field of protozoology.

This year we are inaugurating a new session in our meeting. One of the round tables was organized and will be presented by Early Career Researchers. Through this initiative we are encouraging these researchers to take an active role in our society and to showcase their work and career in an excellency driven environment. 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. 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 is going through recently, guaranteeing the quality and international recognition of the meeting. We would also like to acknowledge the financial support received from CAPES, CNPQ, FAPESP, FIOCRUZ 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, August 22nd, 2022

Luís Carlos Crocco Afonso

President of SBPz

FOTO DA CAPA/COVER BOOK

Molecular Biology approaches to understand *Leishmania.*

A phylogenetic tree is a graph of relationships. Each entity is connected by branches to "common ancestors", which are hypothetical entities indicating the same origin. In this way, the root entity occupies a special place, from where the tree branches emerge. This graph represents the evolution of *Leishmania* (along to other Tryps) studies in our lab. **Gene characterization** is the root or the special entity in our graph, that is, the origin of everything of what the lab does. After gene characterization we have two big sides, one more related to pathogen **Identification** of species that lead to **Diagnosis** possibility and culminate with the establishment of **Phylogenetic** relationships; and another, more related to the physiology of these organisms, showing how **Gene expression** occurs, and then exploring the **Host-parasite interactions** in the infection processes.

Dra. Lucile M. Floeter-Winter

FINANCIAL SUPPORT

AUGUST 22nd, 2022

14h30 – 15h30 | **VIRTUAL POSTER SESSION** **Room Google Meet**
Discussion

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

17h00 – 18h00 | **OPENING CONFERENCE** **ROOM A**
SAMUEL PESSOA AWARD

CHAIR: Jeffrey Shaw

Molecular Biology approaches to understand *Leishmania*
Lucile Maria Floeter-Winter *(USP-IB, São Paulo, SP, Brazil)*

18h00 – 19h30 | **BRAINSTORM** **Room A**

Chair: Ariel M. Silber

Impatient science and academic quantophrenia: reflections on modern scientific culture
Marcus Oliveira *(UFRJ, Rio de Janeiro, RJ, Brazil)*

19h00 – 20h30 | **DINNER**

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

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

Chairs: Milton Oliveira and Maria Fernanda Laranjeira-Silva

HP01 – Gasdermin-D activation in response to Leishmania infection induce a transient cell permeabilization to promote NLRP3 activation and host resistance to infection
Keyla Santos Guedes De Sá

HP02 – Technological development of a new vaccine candidate against canine visceral leishmaniasis caused by *Leishmania infantum.*
Otoni Alves De Oliveira Melo Junior

HP03 – TNFR1, the maestro of inflammatory response to infection with *Leishmania amazonensis*
Leonardo Gomes Vaz

PV01 – Identification and testing of candidates for the development of leishmaniasis Transmission Blocking Vaccines
Thais Lemos-Silva

HP05 – Glycoconjugates (LPG/GIPLS) from dermotropic amazonian leishmania species displays interspecies variations in their biochemical and functional properties in C57BL/6 macrophages
Rodrigo Pedro Pinto Soares

HP06 – Congenital *Toxoplasma gondii* infection affects retinal proliferation and differentiation in mice.
Viviane Souza De Campos

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

Chairs: Dan Lahr and Juliana Aoki

TB01 – Expanding the arsenal against leishmaniasis: novel tamoxifen/clemastine chimera as potential antileishmanials
Victor De Sousa Agostino

PV02 – Glucose Metabolism is Essential to Support Heme-Induced Epimastigotes Proliferation of *Trypanosoma cruzi*
Carolina Silva Dias Vieira

TB02 – Effect of apigenin in vitro and in vivo in *Leishmania infantum.*
Yago Sousa Dos Santos Emiliano

TB03 – Effect of MSS as an alternative therapy for the treatment of leishmaniasis
Gabriella Gonçalves Ozório

TB04 – 2'-hydroxyflavanone effects against wild type and antimony-resistant *L. infantum*, toxicity and pharmacokinetics
Luiza Gervazoni Ferreira De Oliveira

TB05 – Innovative microfluidic device to synthesize amphotericin B-loaded polymeric nanoparticles for cutaneous leishmaniasis treatment
Giovanna Cersósimo Nader Mota

TB06 – Chalcone nanocrystals for oral treatment of cutaneous leishmaniasis
Maria Paula Gonçalves Borsodi

TB07 – MMV Pandemic box in vitro screening identifies new compounds highly active against the tachyzoite stage of *Toxoplasma gondii*
Mike Dos Santos

HP09 – Modifications in lipid metabolism of swiss mice infected with *Toxoplasma gondii*.
Samara Valeria Delgado Andrade

11h00 – 11h30 | **Coffee break**

11h30 – 12h10 | **Conference 01** **ROOM A**
Chair: Santuza M. Ribeiro Teixeira

CO-01-Organization and dynamics of the *Trypanosoma cruzi* genome
Carlos Robello – *(Institute Pasteur – Montevideo, Uruguay)*

12h30 – 14h30 | **Lunch**

13h30 – 14h30 | **Reunião do Conselho**

14h30 – 16h30 | **Round-Table** **ROOM A**
RT.01 – Translational Biology
Chair: Luzia Helena Carvalho

RT01-01 - Role of miR146a-5p in regulating macrophage plasticity during *Leishmania donovani* infection
Nahid Ali *(Indian Institute of Chemical Biology, India)* | Virtual

RT01-02 - From sampling procedures to parasite identification: experiences on diagnosing leishmaniases through real time PCR / HRM
Ricardo Zampieri *(USP, São Paulo, SP, Brazil)*

RT01-03 - Exploring old and new compounds in the search for new treatments for acute and chronic Toxoplasmosis
Érica Martins Duarte *(UFMG, Belo Horizonte, MG, Brazil)*

RT01-04 - Interferon-Gamma and Mitochondrial dysfunction in the pathogenesis of Chagas disease cardiomyopathy: therapeutic targets
Edecio Cunha Neto *(USP, São Paulo, SP, Brazil)*

14h30 – 16h30 | **Round-Table** **ROOM B**
RT.02 – Interactions of pathogenic trypanosomatids with their hosts
Chair: Edmundo C. Grisard

RT02-01 - The Antioxidant systems of *Leishmania* and their role in parasite infectivity: a tale of fascinating enzymes
Ana Tomaz *(Universidade do Porto, Lisboa)*

RT02-02 Studies on paromomycin resistance in *Leishmania amazonensis*
Adriano Capellazzo Coelho *(UNICAMP, Campinas, SP, Brazil)*

RT02-03 - *Trypanosoma cruzi* modulates host transcription and splicing machinery during infection
Munira Baqui *(USP, Ribeirão Preto, SP, Brazil)*

RT02-04 - New insights regarding the regulation of translation in trypanosomatids derived from the study of two eIF4F-like initiation complexes
Oswaldo Pompílio de Melo Neto *(Fiocruz, Recife, PE, Brazil)*

16h30 – 17h00 | **Coffee break**

17h00 – 17h30 | **Conferences 02** **ROOM A**
Chair: Ariel Silber

CO.02 – Treating Latent Infection: Can We Kill Parasites in their Sleep?
Willian J. Sulivan *(Indiana University School of Medicine, USA)*
Virtual

17h00 – 17h30 | **Conferences 03** **ROOM B**
Chair: Narcisa Cunha e Silva

CO.03 – *Trypanosoma cruzi* infection causes cellular stress and a senescence-like phenotype in fibroblasts
Alessandra Filardy *(UFRJ, Rio de Janeiro, RJ, Brazil)*

17h30 – 18h00 | **Conferences 04** **ROOM A**
Chair: Yara M. Traub-Cseko

CO.04 – The role of the kynurenine pathway at the host-microbiota interface in blood-feeding insects
Pedro Lagerblad Oliveira *(UFRJ, Rio de Janeiro, RJ, Brazil)*

17h30 – 18h00 | **Conferences 05** **ROOM B**
Chair: Julio Scharfstein

CO.05 – Applying CRISPR/CAS9 to study gene function in *Trypanosoma cruzi:* insights into the function of peptidases, inhibitors, and protein kinases
Ana Paula Cabral Lima *(UFRJ, Rio de Janeiro, RJ, Brazil)*

AUGUST 23rd, 2022

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

19h00 – 20h30 | **Dinner**

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

09h00 – 11h00 | **Oral Presentations** **ROOM A**
OP.03

Chair: Renata Tonelli and Osvaldo Pompílio

PV05 – Development of a computer visualization tool for the morphological classification of Free-Living Amoebas (FLAs)
Matheus Issa

PV06 – Updated phylogenomic reconstruction of amoebozoan testate amoebae
Daniel José Galafasse Lahr

PV07 – Is there a linker histone in *Toxoplasma gondii*?
Vanessa Rossini Severo

PV08 – The genetic knockout of pyrroline-5-carboxilate synthetase and pyrroline-5-carboxylate reductase reveals a shortcut in the glutamate-proline pathway *in Leishmania braziliensis*
Gustavo Daniel Campagnaro

PV09 – Identification of genes involved in synthesis of bioactive phospholipids and molecular analysis of a platelet activating factor acetylhydrolase (paf-ah) of *Trypanosoma cruzi*
Felipe Soares Coelho

PV10 – Does Leishmania ATM play a role in DNA repair and genome plasticity?
Jennifer Ann Black

HP10 – Modulation of the Host Nuclear Compartment by *Trypanosoma cruzi* Uncovers Effects on Host Transcription and Splicing Machinery
Camila Gachet De Castro

09h00 – 11h00 | **Oral Presentations** **ROOM B**
OP.04

Chair: Patricia Fampa and Renato DaMatta

HP11 – Mechanisms of FAZ assembly in *Trypanosoma cruzi*
Normanda Souza Melo

PV11 – Dynamics of TcRab5 isoforms in *Trypanosoma cruzi* epimastigotes
Alexia Achilles Amaral

HP13 – Physical exercise protects mice from muscular and neural pathology after *Toxoplasma gondii* infection
Paloma De Carvalho Vieira

HP15 – Profiling of angiogenic factors released by *Toxoplasma gondii* cyst-bearing cortical neurons
Leonardo Leal De Castro

HP16 – Characterization of regulation mechanisms involved in llchit1 expression, a

midgut-specific chitinase of *Lutzomyia longipalpis*, the main vector of american visceral leishmaniasis.
Ana Carolina Pedro Santos Ribeiro

HP17 – High-dimensional flow cytometry to evaluate CD4+ T cell heterogeneity during Chagas cardiomyopathy
Gregório Guilherme Almeida

HP18 – Role of P2x7 purinergic molecule in response to *Trypanosoma cruzi* in the acute phase of cardiac infection.
Joaquim Teixeira Xavier Junior

11h00 – 11h30 | **Coffee break**

11h30 – 12h00 | **Conference 06** **ROOM A**
Chair: Elvira M. Saraiva

CO.06 – Extracellular vesicles released from *Leishmania* infected cells (LiEVs); Biogenesis and putative functions in visceral leishmaniasis
Peter Kima *(University of Florida,USA)*

12h00 – 12h30 | **Conferences 07** **ROOM A**
Chair: Julia P. C. da Cunha

CO.07 – Single-molecule analysis of DNA replication in the binucleated *Giardia lamblia* trophozoite
Renata Tonelli *(UNIFESP, Diadema, SP, Brazil)*

12h00 – 12h30 | **Conferences 08** **ROOM B**
Chair: Lucile Floeter-Winter

CO.08 – Iron Metabolism at the Leishmania-Host Interface
Maria Fernanda Laranjeira-Silva *(USP-IB, São Paulo, SP, Brazil)*

12h30 – 14h30 | **Lunch**

14h30 – 16h30 | **Round-Table** **ROOM A**
RT.03 – Host-Parasite Relashionship
Chair: Luís Afonso

RT03-1 - In vivo CRISPR screens identify GRA12 as a transcendent virulence factor of *Toxoplasma gondii*, including in highly virulent South American strains
Francesca Torelli *(The Francis Crick Institute, UK)* Virtual

RT03-2 - Leishmania-Induced Dendritic Cell Migration and Its Potential Contribution to Parasite Dissemination
Juliana Perrone Bezerra de Menezes *(Fiocruz, Salvador, BA, Brazil)*

RT03-3 - Innate immunity in the vasculature and the CNS during *Toxoplasma gondii* infection
Melissa Lodoen (UCI, Irvine, USA)

RT03-4 - Cell invasion by Leishmania infective forms: unveiling hidden routes of infection and their possible impacts
Thiago de Castro *(UFMG, Belo Horizonte, MG, Brazil)*

14h30 – 16h30 | **Round-Table** **ROOM B**
RT.04 – Next Generation (not sequence!) of Protozoologists
Chairs: Carolina Catta Preta, Maria Fernanda Laranjeira-Silva

RT04-1 - Regulation of Transcription in *Leishmania* by Bromodomain Factor 5 and the CRKT complex
Nathaniel Jones *(YORK University, UK)* Virtual

RT04-2 - *Giardia duodenalis* reshapes intestinal mucosal immunity to prevent tissue damage and attenuate *Toxoplasma gondii*-driven inflammation
Aline Sardinha da Silva *(NIAID, NIH, USA)* | Virtual

RT04-3 - Disruption of a single IP6K allele alters cell morphology and leads part of the *Trypanosoma cruzi* population to quiescence
Marcelo Santos da Silva *(UNESP, Botucatu, SP, Brazil)*

RT04-4- *T*ranscriptomic analysis revealed a conserved protein regulated by arginase activity in *Leishmania amazonensis*
Juliana Ide Aoki *(USP, São Paulo, SP, Brazil)*

16h30 – 17h00 | **Coffee-Break**

17h00 – 17h30 | **Conference 09** **ROOM A**
Chair: Marcia Attias
CO.09 – Cell cycle control by ApiAP2 transcription factors in the apicomplexan parasite *Toxoplasma gondii*
Mathieu Gissot *(Institut Pasteur de Lille, France)*

17h00 – 17h30 | **Conference 10** **ROOM B**
Chair: Camila Indiani

CO.10 – 10-Year Follow-up study of Plasmodium vivax DBPII immune response in the Brazilian Amazon
Luzia Helena Carvalho *(FIOCRUZ – Belo Horizonte – MG – Brazil)*

17h30 – 18h30 | **Closing Conference** **ROOM A**
Chair: Maria Carolina Elias Sabbaga

CC - Endosymbiosis in Trypanosomatids and Cell Evolution: Mutual Benefits of Living Together
Maria Cristina Machado Motta *(UFRJ – Rio de Janeiro – RJ – Brazil)*

19h00 – 20h30 | **Dinner**

21h30 – 22h30 | **Closing, Remarks and Awards** **ROOM A**

SPC-1 - **Molecular Biology approaches to understand *Leishmania***

FLOETER-WINTER, L.; AOKI, J.; LARANJEIRA-SILVA, M.F.; ZAMPIERI, R.A..
DEPARTAMENTO DE FISIOLOGIA, INSTITUTO DE BIOCIÊNCIAS, USP, SÃO PAULO - SP - BRA.
E-mail: lucile@ib.usp.br

The purpose of this lecture is to present the paths of a research line, which aims to understand how the protozoan *Leishmania* maintains itself in nature and how it establishes relationships with its hosts. We chose to show these paths as branches of a “tree”, representing the relationships of the “hypothetical entities”. The “root of the tree” occupies a special place since it is the source entity. Thus, we intend to show how “gene characterization” represents the origin of research lines of our lab and how it evolved in two branches: one for the identification of the pathogen, linked to the diagnosis and phylogeny of these parasites, and another related to the physiology of these organisms and their interaction with the hosts.

We started with the characterization of the ribosomal cistron (rDNA), which contains the sequences that will be exclusively transcribed by RNA Polymerase I (RNA polI) to originate RNAs that constitute the ribosome. In this characterization, we used the evolutionary conserved characteristic of the rDNA to search for regions that could be discriminatory among the species of *Leishmania* [1] [2]. However, the SSU rRNA sequence (RNA of the small subunit of the ribosome) is highly conserved and so the desired discrimination was not achieved for the species level. The same was true for the establishment of phylogenetic relationships, which generated unresolved trees. Nevertheless, using information from the SSU rRNA sequence, we confirmed that *L. tarentolae*, a species that infects lizards, belongs to a sub-genus previously identified as *L. (Sauroleishmania)* [3].

Using the rDNA gene characterization, we went to the other branch of our “tree” to study the regulation of rRNA transcription, determining the promoter region of RNA polI of some *Leishmania* species. The functional analysis of RNA polI promoter regions, through transient transfections of constructs containing a reporter gene under promoter region control, showed that RNA polI recognition is species-specific [4]. Additionally, we observed that heterologous RNA polI promoter was more active than its homolog, leading us to propose the existence of a species-specific repressor controlling rDNA transcription [5].

Since discrimination and identification of species was still our goal for later establishment of a diagnostic method using DNA, we moved back to the root of our “tree” to search for other target sequences. We identified Glucose-6-phosphate dehydrogenase (G6PD) coding region that allowed the discrimination of *L. (Viannia) braziliensis* from other *Leishmania* spp. [6] and provided the tool to identify its mammalian reservoir [7]. But we still have not come up with a robust target for species identification. Other sequences were tested, and finally, using the *hsp70* sequence as target in a PCR-HRM (Polymerase Chain Reaction coupled to High Resolution Melting) analysis, we describe a protocol that allowed the identification of all species that circulate in a given territory (Americas or Eurasia) [8]. This protocol was transposed from research benchtop to patient’s samples allowing epidemiological studies and an individual physician conduct in face to patient's prognosis [9].

In our search for species-specific targets, we characterized the arginase coding-gene. Previously, Prof. Erney Camargo’s group demonstrated how urea cycle enzymes have different expression profiles among trypanosomatids genera with *Leishmania* genus expressing arginase, an important enzyme of the urea cycle. Our study indicated that arginase gene sequence was not a good discriminatory target, however, moving to the other branch of the "tree", the arginase gene characterization of *L. (L.) amazonensis* allowed the heterologous expression of the active enzyme and its biochemical and biophysical characterization [10] [11]. With the purified recombinant enzyme, we obtained an anti-arginase polyclonal serum used to determine its localization at the glycosome, trypanosomatids’ exclusive organelle, confirming what had already been predicted by the presence of the SKL-addressing signal at its C-terminal [12].

The next step comprised the understanding of physiological role of arginase and showed its importance in the infection and maintenance of the parasite in the host cell, obtaining a *L. (L.) amazonensis* arginase knockout parasite [12]. We were able to obtain this knockout mutant only under culture supplementation with putrescine, what indicates the essential role of arginase in the synthesis of polyamines for parasite replication and supports arginase potential as a therapeutic target [13]. A more detailed study of the physiological role of arginase was obtained using the null arginase knockout in “omics” approaches, to study the interaction of the parasite with its host. We obtained the transcriptomic [14] and metabolomic [15] profiles of wild-type and knockout parasites and the data allowed an improved understanding of metabolic relationships and regulation of the expression of genes related to the polyamine pathway [16], as well as with virulence factors such as parasite recognition, growth and differentiation [17]. We also identified a possible salvation pathway, which should be considered for the proposed use of inhibitors of parasite’s arginase as therapeutics.

Considering the pathways involved in arginine metabolism and its potential role in host inflammatory processes and immune response, the transcriptomic [18] [19] and metabolomic [20] [21] profiles of BALB/c or C57BL/6 macrophages, infected with wild-type or arginase *L. amazonensis* knockout, indicated the importance of the host genetic background for the infection progression.

In another approach to study the host-parasite interaction, we used a panel of 84 miRNAs to determine the miRNA's profile of infected macrophage [22]. This profile is involved in the macrophage response to infection and, in some way, to parasite's arginase activity. The miRNAs we found in the study were also found in the plasma of *Leishmania*-infected patient [23] [24]. Furthermore, we demonstrated the role of mice miRNAs in regulating NOS2, an enzyme that also uses arginine as a substrate for the synthesis of NO, a potent microbicide. It is interesting to note that the human NOS2 presents a mutation in its mRNA 3'UTR, indicating that the miRNA we identified cannot act in this mRNA [25].

L-arginine, the arginase's substrate, is also essential for *Leishmania* survival. The uptake of arginine from the environment, either from the insect or the mammalian macrophage, is crucial for the parasite. We characterized the gene that encodes Amino Acid Permease 3 (AAP3), the main arginine transporter. We identified two copies of this gene on chromosome 30 and showed that one copy is regulated by extra or intracellular arginine levels [15], changes in temperature and/or pH [26], parameters that naturally change when the parasite passes from one host to another. For this gene, we started characterizing its physiological role and later we showed its species discriminatory potential with the same PCR-HRM approach [27].

Concerning the identification/diagnosis "branch", we are currently exploring the possibility of associating different targets, such as *hsp70* and *aap3*, to obtain an improved identification test for the development of an identification kit, in a multilocus approach. As well as, new genes identified from the "omics" data, are being characterized for their physiological role in studies that can indicate potential chemotherapeutic targets.

Taken together, this "phylogenetic tree" represents the evolution along the years of how gene characterization became a solid root with well-established branches.

References

1. Uliana et al. Exp Parasitol, 1991. **72**(2): p. 157-63.
2. Uliana et al. J Eukaryot Microbiol, 1994. **41**(4): p. 324-30.
3. Briones et al. Mol Biochem Parasitol, 1992. **53**(1-2): p. 121-7.
4. Uliana et al. Mol Biochem Parasitol, 1996. **76**(1-2): p. 245-55.
5. Stempliuk and Floeter-Winter Int J Parasitol, 2002. **32**(4): p. 437-47.
6. Castilho et al. J Clin Microbiol, 2003. **41**(2): p. 540-6.
7. Brandao-Filho et al. Trans R Soc Trop Med Hyg, 2003. **97**(3): p. 291-6.
8. Zampieri et al.PLoS Negl Trop Dis, 2016. **10**(2): p. e0004485.
9. Veasey et al. An Bras Dermatol, 2020. **95**(4): p. 459-468.
10. da Silva ER et al. Int J Parasitol, 2002. **32**(6): p. 727-37.
11. da Silva ER et al. Mol Biochem Parasitol, 2008. **159**(2): p. 104-11.
12. da Silva MF et al. PLoS One, 2012. **7**(3): p. e34022.
13. da Silva MF and Floeter-Winter Subcell Biochem, 2014. **74**: p. 103-17.
14. Aoki et al. PLoS Negl Trop Dis, 2017. **11**(10): p. e0006026.
15. Castilho-Martins et al. Electrophoresis, 2015. **36**(18): p. 2314-2323.
16. Muxel et al. Front Microbiol, 2017. **8**: p. 2682.
17. Aoki. et al. Curr Opin Microbiol, 2019. **52**: p. 110-115.
18. Aoki et al. Microb Genom, 2020. **6**(9).
19. Aoki et al. Sci Rep, 2019. **9**(1): p. 19841.
20. Muxel et al. Int J Mol Sci, 2019. **20**(24).
21. Mamani-Huanca et al. Int J Mol Sci, 2021. **22**(13).
22. Muxel et al. Sci Rep, 2017. **7**: p. 44141.
23. Ramos-Sanchez et al. Front Cell Infect Microbiol, 2022. **12**: p. 826039.
24. Souza et al. Front Cell Infect Microbiol, 2021. **11**: p. 687647.
25. Fernandes et al. in preparation.
26. Aoki et al. PLoS Negl Trop Dis, 2017. **11**(10): p. e0006025.
27. Muller et al.Parasit Vectors, 2018. **11**(1): p. 421.

CO-01 - **Organization and dynamics of the *Trypanosoma cruzi* genome**

ROBELLO, C.A..
INSTITUT PASTEUR DE MONTEVIDEO & FACULTAD DE MEDICINA, MONTEVIDEO - URY
E-mail: robello@pasteur.edu.uy

Trypanosoma cruzi is able to invade almost any kind of cell, freely circulate in the blood or extracellular matrix, pass through the digestive tract of its insect vector and survive after being eliminated in faeces. This stressful lifestyle strongly requires fine regulation of gene expression, which in turn is reflected in its genome organization. Elucidation of the *tritryps* genomes was already representative that the genome of *T. cruzi* had taken particular evolutionary paths: while the genomes of *T. brucei* and *L. major* allowed in-depth comparative studies at the level of synteny and chromosomes, the genome of *T. cruzi* remained highly fragmented due to an expansive effect that results in the presence of multicopy genes. In particular, the surface proteins, which play crucial roles in the invasion, infection and modulation of the immune system, constitute the most expanded families, being encoded by hundreds - and more than a thousand in some cases- of genes. For a long time, it was thought that these genes were located in subtelomeric regions, as occurs in African trypanosomes, which would give them greater reorganisation/recombination capacity. However, a detailed analysis of the nuclear genome of several strains by using long-read sequencing methods led us to the observation that in *T. cruzi* the genome is organized into two clearly differentiated compartments: core and disruptive. The core compartment is composed of conserved genes either from known function or not (e.g.: hypothetical proteins), and they exhibit a high degree of synteny with the rest of the trypanosomatids. In opposition, the disruptive compartment is composed mainly of *transialidase*, *mucin* and *masp* genes and, as is known, synteny is lost or, in other words, synteny disruption occurs.
Long read sequencing allowed chromosome level assembly, and there are chromosomes composed completely for only one compartment, being most of them "mixed" chromosomes, but with a predominance of one of the compartments. When the comparison is made between lineages of *T. cruzi*, also disruption of synteny takes place, meaning that chromosomes predominantly disruptive cannot be numbered indistinctly, so we propose a new nomenclature for them. In addition, this organization is not just a feature of the genome architecture, but significant differences are found between compartments in chromatin organization, intrachromosomal interactions, nucleosome positioning, and methylation patterns, and these differences directly affect stage-specific gene expression levels. **Keywords:** Trypanosoma cruzi;Genomics;Epigenetics.

CO-02 - **Treating Latent Infection: Can We Kill Parasites in Their Sleep?**

SULLIVAN, W.J..
INDIANA UNIVERSITY SCHOOL OF MEDICINE, INDIANAPOLIS - USA.
E-mail: wjsulliv@iu.edu

The ability of pathogenic protozoa to undergo differentiation into latent forms is critical to transmission and pathogenesis. Dormant stages of parasitic infection can lead to chronic disease that is intractable to current drug therapies. Here, we present evidence that a stress signaling pathway that mediates control of protein synthesis drives the formation of latency stages. We show that the development of latent stages in *Toxoplasma gondii* and *Plasmodium falciparum* is accompanied by the phosphorylation of eukaryotic initiation factor-2 (eIF2), which prompts changes in translation that produce factors to reprogram the genome. In *T. gondii*, replicating tachyzoites differentiate into latent bradyzoites that persist in the host and give rise to life-threating episodes of reactivated infection. The conversion to bradyzoites is triggered through TgIF2 phosphorylation by a series of eIF2 kinases that recognize different types of cellular stress. We show that pharmacological inhibition of one of these eIF2 kinases, an essential PERK orthologue, inhibits parasite replication and bradyzoite development in vitro and can subvert artemisinin-induced latency in *P. falciparum*. Furthermore, we find that inhibitors of eIF2 dephosphorylation, such as guanabenz, have anti-parasitic activity that includes destruction of bradyzoite cysts in vitro and in mouse models of latent toxoplasmosis. The discovery of a drug that dramatically decreases brain cyst counts allowed us to examine whether the number of brain cysts is associated with well-documented behavioral changes in rodents harboring latent toxoplasmosis. Unexpectedly, we found that brain cyst counts do not correlate with changes in host behavior; rather, behavior changes are associated with neuroinflammation. Altogether, these findings underscore the relevance of translational control through eIF2 as a key switch between proliferation and latency, and holds great promise to be exploited chemotherapeutically. **Supported by:**NIH **Keywords:** Toxoplasma;translation ;latency.

CO-03 - *Trypanosoma cruzi* infection causes cellular stress and a senescence-like phenotype in fibroblasts

FILARDY, A.D..
IMPG - UFRJ, RJ - BRA.
E-mail: filardy@biof.ufrj.br

Trypanosoma cruzi infects and replicates within a wide variety of immune and non-immune cells. Here, we investigated early cellular responses induced in NIH-3T3 fibroblasts upon infection with trypomastigote forms of *T. cruzi*. We show that fibroblasts were susceptible to *T. cruzi* infection and started to release trypomastigotes to the culture medium after 4 days of infection. Also, we found that *T. cruzi* infection reduced the number of fibroblasts in 3 day-cell cultures, by altering fibroblast proliferation. Infected fibroblasts displayed distinctive phenotypic alterations, including enlarged and flattened morphology with a nuclei accumulation of senescence-associated heterochromatin foci (SAHF). In addition, infection induced an overexpression of the enzyme Senescence-Associated β-galactosidase (SA-β-gal), an activation marker of the cellular senescence program, as well as the production of cytokines and chemokines involved with the senescence-associated secretory phenotype (SASP) such as IL-6, TNF-α, IL-1β, and MCP-1. Infected fibroblasts released increased amounts of stress-associated factors nitric oxide (NO) and reactive oxygen species (ROS), and the treatment with antioxidants deferoxamine (DFO) and N-acetylcysteine (NAC) reduced ROS generation, secretion of SASP-related cytokine IL-6, and SA-β-gal activity by infected fibroblasts. Taken together, our data suggest that *T. cruzi* infection triggers a rapid cellular stress response followed by induction of a senescent-like phenotype in NIH-3T3 fibroblasts, enabling them to act as reservoirs of parasites during the early stages of the Chagas disease.

CO-04 - The role of the kynurenine pathway at the host-microbiota interface in blood-feeding insects

DE OLIVEIRA, P.L..
INSTITUTO DE BIOQUÍMICA MÉDICA LEOPOLDO DE MEIS - UFRJ, RJ - BRA
E-mail: pedro@bioqmed.ufrj.br

Hematophagous insects ingest blood meals that are several-fold their weights before feeding. Excluding water, protein content accounts for 85 % of the composition of vertebrate blood, and therefore, digestion releases exceptionally large amounts of free amino acids. We previously showed that the tyrosine degradation pathway is essential to adapt insects to homeostatically handle dietary tyrosine. Here we studied the role of the tryptophan degradation pathway, the kynurenine pathway (KP). In mammals, intermediates of the KP have been ascribed multiple roles, including modulation of host/microbiome symbiosis. Enzymes of the KP are highly induced in *Aedes aegypti* midgut after a blood meal. We analyzed the phenotypes of a natural kynurenine hydroxylase (*Kh*) mutant in *Aedes aegypti* and CRISPR/Cas9 engineered mutants in *Anopheles stephensi* and *Culex quinquefasciatus*. All species showed a marked loss of fitness after feeding a blood meal. Aedes and Anopheline mosquitoes present different levels of severity, from reduced lifespan and fertility to a compromised midgut permeability barrier function in *An. stephensi*. Kh^- displayed an increase in microbiota when compared to kh^+ insects, without increased expression of canonical immune genes. Metagenomic analysis of *A. aegypti* from Kh mutants and wild type midgut, however, showed relative conservation of relative prevalence of most abundant species, suggesting an effect on microbial community size. Additionally, life span/mortality effects were rescued by antibiotic treatment, revealing a causative role for intestinal dysbiosis. Feeding with xanthurenic acid (XA), one of the KP end products, rescued lethality in both species of mosquitos and limited microbiota expansion in *Ae. aegypti*. These data reveal a unique role of the KP and XA in the regulation of the host/microbiota interface. A role for the aryl hydrocarbon receptor pathway as a downstream target of the KP was investigated.
Supported by:CNPq, FAPERJ **Keywords:** Aedes aegypti;Kynurenine pathway;microbiota.

CO-05 - **APPLYING CRISPR/CAS9 TO STUDY GENE FUNCTION IN *Trypanosoma cruzi*: INSIGHTS INTO THE FUNCTION OF PEPTIDASES, INHIBITORS, AND PROTEIN KINASES**

COSTA, T.F.R.; GOUNDRY, A.L.; LIMA, A.P.C.D.A..
INSTITUTO DE BIOFISICA CARLOS CHAGAS FILHO, UFRJ, RJ - BRA
E-mail: anapaula@biof.ufrj.br

The study of gene function in pathogenic protozoa is a fundamental step to understand parasite biology, its interplay with the host, mechanisms of pathogenesis, and to identify new drug targets. While sophisticated genetic tools are widely used in *Leishmania* and *Trypanosoma brucei*, the genetic manipulation of *T. cruzi* has proved to be more time-consuming and less efficient. As a result, from 1990-2018, gene deletion attempts had only been made for 36 *T. cruzi* genes [1]. Lately, the use of CRISPR/Cas9 has led to a remarkable enhancement in the timing and efficacy of genetic manipulation of *T. cruzi* [2-5]. We have adopted this approach to investigate gene function in *T. cruzi* Dm28 and here, we will describe our findings on the roles of the ecotin-like inhibitor of serine peptidases, ISP2, cathepsin B (CATB), and some protein kinases. We generated ISP2-null mutants (Δ*isp*2) in *T. cruzi* using CRISPR/Cas9, and Δ*isp*2 epimastigotes were more susceptible to lysis by human serum than the parental line or transgenic lines re-expressing ISP2. The Δ*isp*2 tissue culture trypomastigotes (TCTs), were more infective to human muscle cells *in vitro*, which was reverted by addition of camostate mesylate, suggesting that epitheliasin (TMPRSS2) is the target of ISP2. We found that increased host cell invasion is mediated through the crosstalk between the Protease-Activated Receptor 2 (PAR2) and Toll-like Receptor 4 (TLR4), confirmed by siRNA of PAR2, TLR4, or TMPRSS2. Furthermore, Δ*isp*2 induced greater tissue edema in the footpad of BALB/c mice and higher levels of KC, MCP1, TNFα, and IFNγ in the spleen. We propose that ISP2 contributes to protect *T. cruzi* from the antimicrobial effects of human serum and to prevent the engagement of inflammatory receptors in host cells, resulting in the modulation of host cell invasion and decreasing inflammation during acute infection. With regards to the cysteine peptidase cathepsin B, RNAi in *T. brucei* had previously shown that a 32% decrease of TbCATB was sufficient to prevent the death of infected mice, suggesting a possible role in infection. We successfully generated TcrCATB-null mutants (Δ*Tcrcatb*) showing that TcrCATB is not essential in *T. cruzi* epimastigotes. Δ*Tcrcatb* epimastigotes grew normally and did not show alterations in the shape and electron density of reservosomes. Δ*Tcrcatb* TCTs were less infective than the parental line, suggesting that this peptidase may play a role in the interaction with the host. We are currently applying CRISPR/Cas9 to investigate protein kinases (PKs), enzymes that play a predominant regulatory role in cell biology. We used transcriptomic data of Dm28c to select PK genes that were upregulated in the infective stages, and also focused on *T. cruzi* unique PKs (absent from *Leishmania* and *T. brucei* genomes). From this, attempts have been made to generate knock-outs for 13 PK genes. Four null mutants have been achieved, while numerous attempts for other genes did not yield null mutants, potentially indicating gene essentiality. We have discovered a *T. cruzi* unique PK that is vital for the differentiation to the metacyclic form, and has thus been named **E**ssential for **D**ifferentiation **K**inase 1 (EDK1). Likewise, *T. cruzi* lacking a unique STE kinase appeared to have impaired metacyclogenesis, and this PK was named EDK2, while another mutant displayed slow growth as epimastigotes. Among the candidates for essentiality, some will be selected for further validation through facilitated null mutant or unforced plasmid shuffle. Taken together, these findings highlight the current *era* of more efficient and timely analysis of gene function in *T. cruzi* to generate new knowledge on parasite biology.

1- Jones NG *et* al., 2018. ACS Infect Dis. 4(4):467-477
2- Burle-Caldas GA, *et* al, 2018. Int J Parasitol. 48(8):591-596
3 - Lander N, *et* al., 2016. J Biol Chem 291(49):25505–25515
4- Lander N, *et* al., 2019. Methods Mol Biol.1955:61-76
5- Soares Medeiros LC, *et* al., 2017. MBio 8(6)

CO-06 - **Extracellular vesicles released from *Leishmania* infected cells (LiEVs); Biogenesis and putative functions in visceral leishmaniasis**

KIMA, P.. DEPARTMENT OF MICROBIOLOGY AND CELL SCIENCE, UNIVERSITY OF FLORIDA, USA
E-mail: pkima@ufl.edu

Visceral leishmaniasis is a neglected tropical disease that causes significant morbidity and mortality. Iconic images of the distended abdomen of infected individuals due to an enlarged liver and spleen underscores the tissue remodeling in these infections. It is not known how *Leishmania*-derived molecules contribute to this disease presentation. We hypothesized that extracellular vesicles (EVs) released from *Leishmania*-infected cells (LiEVs) that contain parasite derived molecules, induce cellular responses that promote tissue remodeling. Two major mechanisms for exosome cargo loading have been described: the endosomal sorting complex required for transport (ESCRT) pathway and the ESCRT independent pathway ferry molecules that are eventually released from cells in exosomes. Recent studies have begun to elucidate the molecular details of these pathway. There is interest to determine which of these pathways is most important for the loading of molecules from *Leishmania*, which are intravacuolar pathogens. To address this gap in knowledge, we have monitored the intracellular trafficking of *Leishmania donovani* vasohibin (LdVash) in cells expressing fluorophore-tagged components of the ESCRT dependent and ESCRT independent machinery. Our studies have also commenced the investigation of the functional effects of LiEVs. These studies have included evaluation of their capacity to polarize macrophages. I*n vitro* studies thus far have suggested that LiEVs released from *Leishmania*-infected cells have the capacity to induce macrophage polarization to the M2 type. Complimentary *in vivo* studies have also shown more widespread IL4R expression of macrophages in infected tissue, which has provided strong evidence of M2 polarization in *L. donovani* infected tissues, which would ensure parasite persistence.

CO-07 - **Single-molecule analysis of DNA replication in the binucleated *Giardia lamblia* trophozoite**

DA SILVA, M.S.[1]; VITARELLI, M.D.O.[2]; VIALA, V.L.[1]; TSANTARLIS, K.[3]; PIRES, D.D.S.[2]; DE AZEVEDO, I.D.L.M.J.[2]; SABBAGA, M.C.Q.B.E.[2]; TONELLI, R.R.[4].
1. INSTITUTO BUTANTAN/UNESP, SP - BRA; 2. INSTITUTO BUTANTAN, SP - BRA; 3. UNIFESP, SP - BRA; 4. UNIFESP, SP - BRA.
E-mail: renata.tonelli@gmail.com

DNA replication is a highly precise process that must be completed in a timely and accurate manner once each cell cycle. In eukaryotes, DNA replication starts at multiple sites (replication origins) distributed throughout the chromosomes. DNA are RNA synthesis use the same DNA template and can therefore interfere with each other altering gene transcription program and causing replication stress. *Giardia lamblia* trophozoites are binucleated cells that alternate between a tetraploid (2x2N) and an octoploid genome (2x4N) during the S phase of the cell division cycle. Although a previous study suggested that DNA replication in *Giardia* occurs synchronously between the two nuclei, the dynamics of DNA duplication in this organism is poorly understood. Using single-molecule techniques (DNA combing and nanopore-based sequencing followed by D-Nascent analysis) we deeply investigated the spatio and temporal organization of replication units and the progression of replication forks in *G. lamblia* trophozoites. Our data revealed few active origins (between 3-6) on most DNA stretched molecules, showing that chromosomes are fully replicated from a minimum number of origins. Mean Inter-origin distances (IODs) was 335.7 kb and mean replication rate was faster (3.64 ± 1.32 $kb.min^{-1}$) than in any other protozoan parasite analyzed so far. Further, our precise analysis of DNA replication in single trophozoite cells confirmed that both nuclei replicate synchronously (78.49% of cells) with a variation of the stochasticity of replication timing in the other 21.51% of the population. Detection of BrdU incorporated on nascent DNA (D-NAscent) showed that from a total of 11,400 sequenced genes, 5,739 (50.3%) were identified in genomic regions without replication-transcription (RT) collisions collision while 5,661 (49.7%) can be a source of replicative stress by potential head-on RT collisions, meaning that genome organization of *G. lamblia* does not show a co-orientation bias of replication-transcription. Finally, Gene ontology (GO) analysis of genes at sites of HoRT collisions revealed a significant enrichment for GO terms: microtubule-based processes, cell cycle regulation and DNA replication/repair. Most of these genes are key to important cellular processes as cell division, transport of cellular cargo, organelle positioning, and cell migration. Altogether, our combined analyzes provided a whole-genome map of replication dynamics in *Giardia lamblia.*
Supported by:FAPESP and CNPq **Keywords:** Giardia;binucleated cells;DNA replication dynamics.

CO-09 - **Cell cycle control by ApiAP2 transcription factors in the apicomplexan parasite *Toxoplasma gondii*.**

GISSOT, M.. I
NSTITUT PASTEUR DE LILLE - CENTRE D'INFECTION ET D'IMMUNITE DE LILLE, FRA
E-mail: mathieu.gissot@pasteur-lille.fr

All apicomplexan parasites have complex life cycles exhibiting division characterized by a tightly regulated cell cycle control and resulting in the emergence of a various number of daughter parasites. They have evolved efficient and distinctive strategies for intracellular replication where the timing of emergence of the daughter cells, a process termed “budding”, is a decisive element. However, the molecular mechanisms that provide the proper timing of parasite budding remain unknown. Using *Toxoplasma gondii* as a model Apicomplexan, we identified a master regulator that controls the budding process. We show that an ApiAP2 transcription factor, TgAP2IX-5, regulates cell cycle events downstream of centrosome duplication including organelle division and segregation. TgAP2IX-5 binds to the promoter of hundreds of genes and controls the activation of the budding specific cell cycle expression program. TgAP2IX-5 also controls the expression of other ApiAP2 transcription factors, including one that participates in the repression of the developmental switch toward the latent form of the parasite. We are currently investigating the role of these other ApiAP2 transcription factors in controlling the following steps of the cell cycle. **Keywords:** Toxoplasma;cell cycle;apicomplexa.

CO-10 - **10-Year Follow-up study of *Plasmodium vivax* DBPII immune response in the Brazilian Amazon**

CARVALHO, L.H.; KANO, F.S..
INSTITUTO RENÉ RACHOU, FIOCRUZ-MINAS, BH - MG - BRA.
E-mail: lhcarvalho@cpqrr.fiocruz.br

The development of vaccines against *Plasmodium vivax* may be critical in the success of a research agenda to underpin malaria elimination. *Plasmodium vivax* blood stage vaccine strategies have concentrated on region II of Duffy Binding Protein (DBPII) because for reticulocyte invasion most *P.vivax* isolates depends of the interaction between DBPII and Duffy receptor for chemokine (Duffy/DARC).Although DBPII is a major vaccine candidate, we provided evidence that the DBP immune response is strain specific and short lived. While DBPII is weakly immunogenic and induces strain specific immunity, few individuals develop a strain-transcending immune response (“elite responders), indicating that DBPII includes non-polymorphic and less immunogenic epitopes that can be targets for a more broadly inhibitory antibody response. To by-pass DBPII polymorphisms, we have engineered a novel synthetic DBPII-based vaccine (DEKnull-2), which was highly recognized by native Amazonian populations, including the elite responders. Here, we are presenting results of a long-term follow-up to study to characterize the profile of antibody and cellular responses to DEKnull-2 focusing on antigen-specific memory B cells. We are confident that results obtained here may contribute to further improvement of DBPII vaccines that can induce long-term protective immune response. **Supported by:**CNPq, FAPEMIG, NIH-RO1
Keywords: MALARIA;PLASMODIUM VIVAX;VACCINE .

CO-08 - **Iron Metabolism at the Leishmania-Host Interface**

LARANJEIRA-SILVA, M.F..
FEDERAL UNIVERSITY OF ABC, BRA AND UNIVERSITY OF SÃO PAULO, SP - BRA
E-mail: mfernandals@gmail.com

The parasitic protozoa of the Leishmania genus are the agents of the neglected tropical diseases denominated leishmaniases that affect millions of humans around the world, with clinical manifestations ranging from disfiguring cutaneous lesions to visceral disease and death. Therapy remains limited due to toxicity of drugs, poor implementation, and drug resistance. After transmission to a mammalian host by a sand fly vector, intracellular replication within macrophages is critical for leishmaniases pathogenesis. This process requires the acquisition of essential nutrients from the host, such as iron and heme, as Leishmania does not have cytosolic iron storage proteins and lacks the capacity for heme synthesis. Furthermore, both free iron and heme are cytotoxic due to generation of reactive radicals. Leishmania must therefore acquire host heme and iron for survival inside a host environment that restricts availability of these nutrients to the pathogen – a process termed nutritional immunity. Thus, successful colonization requires the pathogen to circumvent
host iron and heme-related defense mechanisms. The study of genes related to iron trafficking in these parasites revealed that the availability of iron plays a central role in the generation of infective parasites. Iron deprivation modulates the expression of several non-characterized genes, providing an excellent opportunity for the identification of additional components of the molecular machinery responsible for iron acquisition, storage, and metabolism in these parasites. So, investigating how these parasites regulate their cytosolic iron concentration to prevent toxicity, we identified and characterized the Leishmania Iron Regulator 1 (LIR1), an iron responsive MFS-type plasma membrane protein with similarity to plant nodulin-like proteins. Consistent with a role in iron efflux, LIR1 deficiency increases parasite sensitivity to iron toxicity and abolishes parasite infectivity. Besides, preliminary data indicate the role of LIR1 in manganese transport and arginase activity. Further analysis of the previously published transcriptome profile of L. amazonensis genes modulated
by iron deprivation is being conducted for identification and characterization of new genes involved in the transport and metabolism of iron, and potentially other transition metals. From this analysis, we identified two genes with predicted glycosomal targeting signals that are currently being studied for elucidation of the mechanisms involved in the metabolism and transport of iron to Leishmania glycosomes. Glycosomes are unique organelles that constitute one of the main differences between parasite and host. These organelles compartmentalize enzymes that use iron as a cofactor, however, the transport of iron into the glycosomes has not yet been characterized. These studies are central for understanding the physiology of these parasites and should reveal novel targets for leishmaniasis therapeutic intervention. **Keywords:** Heme;ion transport;transition metals.

CC-**Endosymbiosis in Trypanosomatids and Cell Evolution: Mutual Benefits of Living Together**

MOTTA, M.C.M..
BIOF-UFRJ, RIO DE JANEIRO - RJ - BRA.
E-mail: motta@biof.ufrj.br

During the last decades, knowledge about the cell biology of trypanosomatids has mostly derived from research on parasitic diseases. Here I will discuss the body of information that emerged from studies of the cell biology of non-pathogenic protozoa, with a particular focus on the metabolic and structural adaptations that symbiotic bacteria and monoxenic trypanosomatids underwent during their coevolution. The study of the cooperative interactions between primitive organisms is essential to understand the mechanisms underlying the increased complexity and diversification of eukaryotic cells. Starting with basic facts, there are currently 20 well-characterized genera in protozoa, of which only 5 include species that are pathogenic to mammals and plants. This implies that monoxenic protozoa, which commonly inhabit in a single host throughout their life cycle, usually insects, comprise the vast majority of currently known trypanosomatid species. Importantly, there are 7 species, belonging to different genera, containing a single symbiotic bacterium in their cytoplasm. One of the central themes of evolutionary biology is to understand how symbiosis contributed to the origin of the organelles of eukaryotic cells. Endosymbiosis in trypanosomatids is a classic example of a mutualistic relationship, since neither the bacterium and the protozoan can live separately. As shown by our group, this coevolutionary process was shaped by morphological and biochemical changes that ensured a harmonious relationship between the bacterium and the trypanosomatid host. Remarkably, the intense metabolic exchange that takes place between both partners has turned the trypanosomatid host into an undemanding cell from a nutritional point of view. In a recent study, we obtained evidences that the intracellular bacterium optimizes the energy metabolism of the trypanosomatid host while inducing changes in the structural arrangement of the mitochondrial DNA contained in the kinetoplast (kDNA), as well as in the cytoskeleton of microtubules. Another example of the mutual benefits of “living together” is the precise synchronization between the division of the symbiont with that of trypanosomatid structures, so that each new protozoan generated always contains a single bacterium. Using the RNAi and CRISPR-Cas9 systems to manipulate gene expression of key trypanosomatid proteins, we recently found that the coordination of cellular and symbiont division is achieved at expense of increased dynamism of microtubules. Now enriched by the full-length sequencing of the genome of both partners, the field of endosymbiosis in trypanosomatids stands itself as an essential branch of research in evolutionary cell biology.
Supported by:FAPERJ and CNPq

RT.01 – Translational Biology

RT01-01 - **Role of miR146a-5p in regulating macrophage plasticity during *Leishmania donovani* infection**

ALI, N..
INFECTIOUS DISEASES & IMMUNOLOGY DIVISION CSIR-INDIAN INSTITUTE OF CHEMICAL BIOLOGY, KOLKATA - IND
E-mail: nali@iicb.res.in

Visceral leishmaniasis (VL) caused by *Leishmania donovani*, is fatal if left untreated. Therapeutics currently used have severe side effects and there is a risk of development of resistance. Thus, identification and characterization of new host directed drug therapy could be a better option. During VL, the parasite induces upregulation of M2 macrophage polarisation genes such as IL-10, arginase 1, etc., concomitantly downregulating M1 marker genes such as 1L-12a, STAT1 and NF-kB for establishing infection in the macrophage. Recently, a role of small non-coding RNAs called microRNAs in regulating such plasticity has been highlighted. However, the mechanistic insight of microRNA regulation and macrophage polarisation during *Leishmania* infection remains elusive. Here, in this study we have undertaken small RNA sequencing of BALB/c mice BMDMs infected with virulent and nonvirulent parasites. We found a differential enrichment of macrophage polarity regulating micro-RNAs including miR146a-5p during infection. miR146a encoding genes have been reported to be regulated by unique epigenetic elements called as super enhancer (SE). To our surprise, we found prolonged expression of miRNA146a-5p along with SE complex such as BRD4, P300 Acetyl transferase and RNA polymerase II during infection. Infection induced miR146a-5p promoted the M2 polarisation of macrophage by targeting TRAF6-IRAK-1-NF-kB signalling axis. Depletion of SE element, bromodomain-containing protein 4 (BRD4), in infected BMDMs, using esiBRD4 RNA showed downregulation of miRNA146a and M2 marker genes such as IL-10 and Arginase 1. Finally, through BRD4 CHIP assay, we confirmed the enhanced occupancy of miR146a-5p enhancer with SE element. Together, we found a unique regulation of M2 macrophage polarity mediator miRNA146a-5p through SE. Our study highlights the miRNA inhibitor-based therapy to overcome the issues of immune suppression which may be further developed for therapeutic interventions.

RT01-02 - **From sampling procedures to parasite identification: experiences on diagnosing leishmaniases through real time PCR / HRM**

ZAMPIERI, R.A.[1]; AOKI, J.[1]; VEASEY, J.V.[2]; LARANJEIRA-SILVA, M.F.[1]; MULLER, K.E.[3]; NERLAND, A.H.[4]; SHAW, J.[1]; FLOETER-WINTER, L.[1].
1. *USP*- SP - BRA; 2. SANTA CASA DE MISERICÓRDIA DE SÃO PAULO, SP - BRA; 3. VESTRE VIKEN HOSPITAL TRUST, DRAMMEN - NOR; 4. UNIVERSITY OF BERGEN, NOR
E-mail: ricardoz@ib.usp.br

The relevance of leishmaniases as a public health problem in several developing countries with around 350 million people at risk of infection and around 2 million new cases every year clearly indicates the need of accurate diagnostic procedures. More than 20 *Leishmania* species are involved in human infections with a wide range of clinical manifestations and, therefore, a precise identification of the parasite is important for clinical diagnosis, treatment and follow-up management. Additionally, diagnostic tests are critical in conducting active surveillance and identifying risk factors.
PCR-based assays are the main approaches among molecular techniques currently used for the diagnosis of leishmaniases. These protocols have increased the sensitivity and specificity compared to those of the conventional approaches based on parasite culture and microscopy. The purity and integrity of DNA samples are factors that strongly influence the results of PCR-based assays. Therefore, choosing suitable procedures for obtaining DNA from patients' biopsies for diagnostic purposes is paramount, from sample collection to extraction methods. Different targets and strategies can be employed in PCR-based assays to detect, quantify and identify *Leishmania* spp. in biological samples. HRM – High Resolution Melting analyses have been described as a useful tool for diagnosing leishmaniases, with all advantages inherent to the real time PCR methodology: target detection / discrimination is achieved by monitoring the accumulation of specific products within a closed tube, with no need for sequencing or gel fractionation,

minimizing risks of environment contamination; it is also a relatively rapid, simple, sensitive, specific and low-cost method.
Here we report over 20 years of experience in diagnosing leishmaniases with the tools available in our laboratory since the advent of PCR, showing which procedures were more accurate from sample collection to the final results of molecular tests. Sampling protocols were systematically evaluated, including storage procedures and DNA purification methods for *Leishmania* spp. detection and quantification in biological samples. The efficiency of three preservation solutions, a phosphate buffer solution, an EDTA buffer solution, and 70% ethanol were compared in combination with three DNA extraction protocols: a commercial silica column kit, salting-out protein precipitation and organic extraction with phenol-chloroform. The resultant DNA was used in real-time PCR assays for the detection and quantification of parasite and host targets. The results of the optimized protocols showed that the DNA extraction method did not influence the test quality, but DNA from samples preserved with the EDTA buffer solution produced higher amounts of target amplicons. Based on these results, we concluded that samples from suspected cases of leishmaniasis to be submitted to molecular diagnostic procedures should be preferentially preserved in EDTA followed by any DNA purification. Exploring the HRM profiles of amplicons from real time PCR assays targeting heat shock protein 70 or amino acid permease 3 coding sequences revealed differences that allowed the discrimination of *Leishmania* species found in the Americas, Eurasia and Africa in samples obtained from standard promastigote culture, naturally infected phlebotomines, experimentally infected mice and clinical human samples, including fresh biopsies, paraffinized samples and smears.
The knowledge accumulated on the bench could be applied in practical situations, in which the precise identification of the parasite was a useful tool for physicians in indicating an appropriate treatment and prognosis. Here we would like to exemplify how the knowledge generated by basic research can be extrapolate from the laboratory routine to the understanding of eco-epidemiological scenarios, planning of public health actions and determining practical clinical outcomes.
***Supported by:*:** FAPESP 2018/23512-0 ***Keywords*:** Leishmania;Diagnosis;DNA.

RT01-03 - **Exploring old and new compounds in the search for new treatments for acute and chronic Toxoplasmosis**

DUARTE, E.D.S.M..
UFMG - MG - BRA.
E-mail: emartinsduarte@gmail.com.br

Toxoplasma gondii is the protozoan responsible for causing toxoplasmosis, a disease commonly associated with congenital malformations, retinochoroiditis, and encephalitis in immunocompromised individuals. It is estimated that 30% of the world's population is infected with *T. gondii*, however, the rates of infection vary according to each region. For example, Brazil is one of the countries with the highest infection rates and it is estimated that at least 50-60% of the population is infected with *T. gondii*. In addition to its high prevalence, Brazil and other South American countries also show a higher incidence of symptomatic cases and sequelae than other regions. Lesions from retinochoroiditis due to congenital toxoplasmosis are more severe and frequent in the Americas than in Europe. In Brazil, ocular toxoplasmosis is the most common cause of childhood blindness and adult posterior uveitis. Despite its high prevalence and importance, treatment for toxoplasmosis is far from ideal. For more than 60 years, the therapy of choice has been based on the administration of the combination of sulfadiazine (SDZ) and pyrimethamine (PYR). Although this combination is generally effective in the acute phase of the disease, it is related to several side effects and is ineffective in the chronic form of the disease. As well as the alternatives available to replace SDZ and PYR are also related to side effects. Indeed some patients show a reduced response to treatment, suggesting the existence of strains with less susceptibility or even resistance to the current treatment drugs. Thus, new compounds or drugs with low toxicity and active against the acute and, especially, the chronic stage of *T. gondii* are of utmost importance. With this objective, our group has been using different strategies such as drug repositioning and the investigation of new molecules to discover new potential chemical entities against acute and chronic toxoplasmosis. ***Supported by:*** CNPq Universal 408964/2018-9; UFMG PRPq ***Keywords*:** Toxoplasma gondii;repositioning;phenanthrolines.

RT01-04 - **Interferon-Gamma and Mitochondrial dysfunction in the pathogenesis of Chagas disease cardiomyopathy: therapeutic targets**

NETO, E.C.[1]; NUNES, J.P.S.[1]; AMEIDA, R.R.[1]; KALIL, J.[1]; CHEVILLARD, C.[2].
1. FACULDADE DE MEDICINA DA USP, SP - BRA; 2. INSERM, MARSELHA - FRA
E-mail: edecunha@usp.br

,
Mitochondria are the central organelles of metabolism, and the main source of ATP. Mitochondrial dysfunction triggers severe disease, including cardiomyopathy, chronic inflammation and neurodegeneration. We will here spotlight the interplay of inflammation and mitochondrial dysfunction in Chagas disease cardiomyopathy (CCC), which has a worse prognosis than other cardiomyopathies. IFNγ -mediated inflammation and mitochondrial dysfunction play a pathogenic role. CCC patients display and increased number of IFNγ-producing T cells in peripheral blood as compared to indeterminate form patients. IFNγ is the most highly expressed cytokine in the CCC heart, and is the top upstream regulator of the transcriptome. Proteomics in CCC myocardium disclosed mitochondrial dysfunction and reduced lipid beta-oxidation pathways.Exome sequencing in families with multiple CCC cases has disclosed heterozygous rare pathogenic variants in mitochondrial and inflammatory genes segregating in CCC cases. IFNγ and TNFα stimulation of AC16 cardiomyocytes recapitulated myocardial oxidative/nitrosative stress and mitochondrial dysfunction found in CCC myocardium acting through the STAT1/NF-kB/NOS2 pathways. Cytokine-stimulated cardiomyocytes further display decreased mitochondrial ATP production and dependency of fatty acid oxidation. Treatment of cardiomyocytes with mitochondria-sparing agonists of AMPK, NRF2 and SIRT1 rescues mitochondrial membrane potential in IFNγ/TNFa-stimulated cells. Our results suggest that IFNγ and TNFa cause direct damage to cardiomyocytes' mitochondria by promoting oxidative and nitrosative stress and impairing energy production pathways, which can act as the second hit on genetically susceptible individuals and induce overt mitochondrial dysfunction with progression to CCC. Treatment with mitochondria-sparing agents might be an approach to ameliorate the progression of CCC. ***Supported by:*** FAPESP 2013/50302-3 ***Keywords*****:** Chagas disease;Interferon-gamma;Mitochondria.

RT.02 – Interactions of pathogenic trypanosomatids with their hosts

RT02-01 - **The Antioxidant systems of *Leishmania* and their role in parasite infectivity: a tale of fascinating enzymes**

TOMAZ, A.M.L.R..
INSTITUTO DE INVESTIGAÇÃO E INOVAÇÃO EM SAÚDE/ICBAS (UNIVERSIDADE DO PORTO), PRT
E-mail: atomas@ibmc.up.pt

In spite of over 100 years of research, infections by *Leishmania* species continue to inflict a heavy burden on human health, affecting approximately 12 million people worldwide and resulting in severe disfigurement, disability and death. To overcome this situation, better treatment regimens are needed. Because these parasites are exposed to oxidants of both endogenous and exogenous origin, and yet they are devoid of catalase and classical glutathione-peroxidase activity, their antioxidant capacity has long been viewed as a possible target of therapy. The fact that such defenses depend on the unique thiol trypanothione, instead of the ubiquitous glutathione, further suggested that the parasite antioxidant systems could be selectively inhibited.
Inspired by the seminal work by Leopold Flohé and colleagues, back in 1997 (Nogoceke et al, Biol Chem, 378:827,1997), our group has been dissecting the mechanisms used by *Leishmania* to reduce hydroperoxides. We focused mainly on the tryparedoxin-tryparedoxin peroxidase systems, investigating their essentiality and role in *Leishmania* infectivity. This talk will provide and overview of our main findings. We will i) discuss the present picture regarding the antioxidant machinery of *Leishmania*, ii) inform on the pertinence of the different enzymes to survival of the parasite disease-causing stage, and discuss which have a higher prospect of being inactivated by drugs. Finally, we will iii) highlight unanticipated aspects of some of the components of the parasite antioxidant machinery as we found them to be much more than mere antioxidant weapons.

RT02-02 - **Studies on paromomycin resistance in *Leishmania amazonensis***

COELHO, A.C.[1]; VERGARA, P.T.[2]; GREIF, G.[3]; SANTOS, Y.[4]; FERREIRA, B.A.[1]; DA SILVA, C.S.[1]; COBB, S.[4]; ROBELLO, C.A.[3]; ALVES, J.M.P.[2]; COSER, E.M.[1].
1. UNICAMP, CAMPINAS - SP - BRA; 2. USP, - SP - BRA; 3. INSTITUT PASTEUR DE MONTEVIDEO, URY; 4. DURHAM UNIVERSITY, DURHAM - GBR
E-mail: ccadriano@gmail.com

Paromomycin (PM) is an aminoglycoside antibiotic used in the treatment of visceral leishmaniasis and also as topical agent against cutaneous leishmaniasis. This drug inhibits the translation machinery, although the mechanism of action is not completely understood in *Leishmania*. Due to the limitations of the treatment specially in Brazil and the potential of PM as an alternative drug, our aim is to identify potential genes associated with PM susceptibility and resistance in *L. amazonensis,* in order to understand the mechanism of action and identify the main targets of PM. To achieve this goal, we have generated resistant lines *in vitro* as promastigotes and intracellular amastigotes. The selection of PM resistant lines was done through three strategies: *in vitro* mutagenesis and stepwise selection in both forms of the parasite. Additionally, we have characterized *L. amazonensis* clinical isolates with differential susceptibility to PM that are intrinsically resistant and susceptible to PM. These resistant and susceptible lines to PM have their whole genome sequenced by next generation sequencing and single nucleotide polymorphisms, mutations, insertions and deletions were identified. Currently, potential genes involved in PM resistance are being functionally validated by gene knockout using CRISPR/Cas9 technology and/or gene overexpression. Among these genes, *CDPK1*, a gene that codes for a protein kinase involved in the control of translation and previously involved in PM resistance in *L. infantum*, was mutated in resistant lines selected by *in vitro* mutagenesis, but not in clinical isolates that are intrinsically resistant to PM. Gene inactivation of *CDPK1* gene confirmed its role in PM resistance in *L. amazonensis.* PM accumulation in isolates with differential susceptibility was evaluated by fluorescence microscopy and flow cytometry, using a fluorescent analog of PM. We found a direct correlation between PM susceptibility and accumulation of this drug in this species, indicating that a transporter may be involved in the resistance phenotype. Moreover, *in vivo* studies in mice infected with isolates susceptible and resistant to PM showed that the effectiveness of this drug is directly correlated with the susceptibility of the parasite *in vitro*. ***Supported by:*** FAPESP: 2016/21161-7; CNPq: 405235/2021-6; UK Research and Innovation: MR/P027989/1
***Keywords*:** Paromomycin;Drug resistance;Leishmania amazonensis.

RT02-03 - ***Trypanosoma cruzi* modulates host transcription and splicing machinery during infection**

BAQUI, M.M.A..
FMRP - SP – BRA - E-mail: munira@fmrp.usp.br

Parasites depend on host factors for every step of their life cycle. During infection, parasite-associated modifications occur to the host cell metabolism and morphology. *Trypanosoma cruzi*, the causative agent of Chagas Disease, lives intracellularly within host cells. However, little is known about the effect of *T. cruzi* infection on the host cell nucleus and nuclear functionality. We recently showed that *T. cruzi* modulates host transcription and splicing machinery in non-professional phagocytic cells during infection. We found that *T. cruzi* regulates host RNA polymerase II (RNAPII) in a time-dependent manner, resulting in a drastic decrease in RNAPII activity. Furthermore, host cell ribonucleoproteins associated with mRNA transcription (hnRNPA1 and A2B1) are downregulated concurrently. We reasoned that *T. cruzi* might hijack the host U2AF35 auxiliary factor, a key regulator for RNA processing, as a strategy to affect the splicing machinery activities directly. Using an adenovirus E1A pre-mRNA splicing reporter, we performed *in vivo* splicing assays revealing that intracellular *T. cruzi* directly modulates host cells by appropriating U2AF35. For the first time, our results provide evidence of a complex and intimate molecular relationship between *T. cruzi* and the host cell nucleus during infection. **Supported by:** FAPESP 2018/03677-5, CAPES, FAEPA
***Keywords*:** Trypanosoma cruzi;Splicing;host-parasite interactions.

RT02-04 - **New insights regarding the regulation of translation in trypanosomatids derived from the study of two eIF4F-like initiation complexes**

NETO, O.P.D.M.[1]; BEZERRA, M.J.R.[1]; DE ASSIS, L.A.[1]; OLIVEIRA, S.S.S.[1]; DE BRITO, A.N.A.L.M.[1]; DE LIMA, G.B.[1]; LIMA, T.D.C.D.C.[2]; CARRINGTON, D.M.[3]; MOURA, D.M.N.[1]; REIS, C.R.D.S.[1].
1. INSTITUTO AGGEU MAGALHÃES/FIOCRUZ, RECIFE - PE - BRA; 2. CENTRO UNIVERSITÁRIO TABOSA DE ALMEIDA, RECIFE - PE - BRA; 3. UNIVERSITY OF CAMBRIDGE, CAMBRIDGE - ENG
E-mail: osvaldo.pompilio@fiocruz.br

Regulating the initiation stage of the mRNA translation, during protein synthesis, is critical for proper regulation of gene expression in most organisms and even more relevant in trypanosomatids, due to their general lack of regulation during mRNA synthesis. Most known mechanisms regulating translation involve the Initiation Factors (eIFs in eukaryotes), with several targeting the eIF4F complex, required for mRNA recognition and ribosome recruitment. eIF4F is formed by the union of eIF4E, the cap binding protein, with the large eIF4G subunit, which binds to several other eIFs and proteins partners. One or two eIF4F complexes are seen in most eukaryotes. Within trypanosomatids, however, five eIF4F-like complexes were identified by us and others, based on distinct eIF4Es and eIF4Gs. Their presence implies specific functions regulating translation and gene expression, but until recently no clear roles for specific complexes were defined. Focusing on the two best studied complexes from *Trypanosoma brucei* and *Leishmania*, based on the EIF4E4/EIF4G3 and EIF4E3/EIF4G4 subunits, we have been able to define distinct protein partners and mRNAs targets for each complex which indicate relevant differences in their mode of action. We found a limited but specific set of RNA binding proteins (RBPs) with EIF4E4/EIF4G3, with the complex preferentially supporting the translation of mRNAs encoding ribosomal proteins. Conserved phosphorylation of EIF4E4 by cell cycle regulated kinases links the regulation of translation of these mRNAs with the trypanosomatid cell-cycle and growth phase. A more diverse set of RBPs and mRNA targets was found associated with EIF4E3/EIF4G4, with EIF4E3 being targeted by post-translational modifications which vary between species and need to be better understood. Our results are compatible with both complexes being impacted by multiple signals which help define the fate of bound mRNAs and require the action of different RBPs and other proteins. ***Supported by***: FACEPE (APQ-1662-2.02/ 15), CNPq (401888/2013-4; 400789/2019-1; 310032/2019-9), CAPES/FACEPE/PNPD (APQ-0876-2.02/16).
Keywords**:** PROTEIN SYNTHESIS;GENE EXPRESSION;REGULATION.

RT.03 – Host-parasite relashionship

RT03-1 - ***In vivo* CRISPR screens identify GRA12 as a transcendent virulence factor of *Toxoplasma gondii*, including in highly virulent South American strains**

TORELLI, F..
THE FRANCIS CRICK INSTITUTE, GBR
E-mail: francesca.torelli@crick.ac.uk

Toxoplasma gondii is a ubiquitous Apicomplexan parasite with an incredibly vast host range that granted it the reputation of most successful parasite on Earth. *Toxoplasma* infects any nucleated cell type and any warm-blooded animal, including one third of the human population. The virulence varies widely, ranging from strains considered mildly pathogenic to virulent ones that caused several outbreaks. Isolates identified in South America (SA), particularly in Brazil, are highly associated with severe clinical symptoms in humans, including vertical transmission and ocular Toxoplasmosis, and even caused fatalities in immunocompetent individuals. The virulence factors specifically responsible for the higher virulence of SA isolates compared to strains from Europe and North America is currently unknown. *Toxoplasma*'s ability to survive within the infected cell and to protect its replicative niche, the parasitophorous vacuole, is secured by proteins secreted from the parasite into the host cell after invasion. The secretome includes dense granules (GRA) and rhoptry proteins (ROP). Recent work

has estimated their number to be over 200, the vast majority of which remains uncharacterised. Most effectors important for parasite survival within the infected cell have been shown to be either host- or parasite strain specific, such as the secreted rhoptry kinase ROP18. However, effector proteins required to colonize multiple if not all hosts, or that are in common between *Toxoplasma* lineages are largely unknown. We performed CRISPR-Cas9 genetic screens targeting all putative secreted proteins to identify factors contributing to parasite survival *in vivo* in 4 *Toxoplasma* strains of different pathogenicity, including the strain VAND which was isolated form a lethal *Toxoplasma* infection in French Guyana. We identified GRA12 as a key protein required for parasite growth in the mouse peritoneum, regardless of the host or the parasite genetic backgrounds. A CRISPR screen in IFNγ-treated murine bone marrow-derived macrophages supports a most critical function of GRA12 for promoting parasite survival within the infected cell. To confirm the results, we engineered a VAND strain that lacks the ability for non-homologous end-joining (Dku80) but retains high virulence *in vivo*. Targeted deletion of *Gra12* in the VANDDku80 strain confirms GRA12 as virulence factor *in vivo* and important to protect *Toxoplasma* from the cell-autonomous immune response, independently from the known strain-specific virulence factors ROP18. Importantly, VANDDku80 is a novel tool for research on the chronic infection, as it retains the capacity to develop tissue cysts both *in vitro* and *in vivo*. Targeted deletion of *Gra12* in other 2 Toxoplasma strains confirms its role as a virulence factor in macrophages of mice, rat and human origin, indicating a parasite strain- and species-transcendent function. Preliminary results show that GRA12 orthologs from the closely related parasites *Hammondia hammondi* and *Neospora caninum* can complement the function of TgGRA12 demonstrating a likely role beyond *Toxoplasma*. GRA12 resides inside the parasitophorous vacuole but affects recruitment of host autophagic markers, such as p62. Pull down of GRA12 identified host proteins involved in known cell autonomous restriction pathways, such as lysosome clearance and autophagy. Current investigation focuses on how GRA12 functions at the host-pathogen interface. In summary, we show that GRA12 is a strain- and species- transcendent virulence factor critical for the cell-autonomous survival of *Toxoplasma*. We also present a novel transgenic parasite strain, VANDDku80, as a research tool to study host-pathogen interaction in clinical strains from South America.

RT03-2 - ***Leishmania*-Induced Dendritic Cell Migration and Its Potential Contribution to Parasite Dissemination**

PAIXÃO, A.R.[1]; SILVA, T.S.[1]; MEDINA, L.S.M.L.S.[2]; PAREDES, B.D.P.B.D.[3]; ARAGÃO, L.[3]; SOUZA, B.[1]; BORGES, V.[1]; SCHRIEFER, A.S.A.[2]; VERAS, P.[1]; BRODSKYN, C.[1]; DE MENEZES, J.P.B.[1].
1. GONÇALO MONIZ INSTITUTE, BA - BRA; 2. FEDERAL UNIVERSITY OF BAHIA, BA - BRA; 3. SÃO RAFAEL HOSPITAL, BA - BRA.
E-mail: julianaperrone@gmail.com

Leishmania, an intracellular parasite species, causes lesions on the skin and in the mucosa and internal organs. The dissemination of infected host cells containing *Leishmania* is crucial to parasite survival and the establishment of infection. Migratory phenomena and the mechanisms underlying the dissemination of *Leishmania*-infected human dendritic cells (hDCs) remain poorly understood. The present study aimed to investigate differences among factors involved in hDC migration by comparing infection with visceral leishmaniasis (VL) induced by *Leishmania infantum* with diverse clinical forms of tegumentary leishmaniasis (TL) induced by *Leishmania braziliensis* or *Leishmania amazonensis* in 2D and 3D environments. Following the infection of hDCs by isolates obtained from patients with different clinical forms of *Leishmania*, the formation of adhesion complexes, actin polymerization, and CCR7 expression were evaluated. We observed increased hDC migration following infection with isolates of *L. infantum* (VL), as well as disseminated (DL) and diffuse (DCL) forms of cutaneous leishmaniasis (CL) caused by *L. braziliensis* and *L. amazonensis*, respectively. Increased expression of proteins involved in adhesion complex formation and actin polymerization, as well as higher CCR7 expression, were seen in hDCs infected with *L. infantum*, DL and DCL isolates. Together, our results suggest that hDCs play an important role in the dissemination of *Leishmania* parasites in the vertebrate host. **Supported by:** CNPq (435536/2018-4), Programa Inova Fiocruz (1131778468), NIH (AI136032) ***Keywords*:** dendritic cell;Leishmania;migration.

RT03-3 - **Innate immunity in the vasculature and the CNS during *Toxoplasma gondii* infection**

LODOEN, M..
UNIVERSITY OF CALIFORNIA - *USA*
E-mail: mlodoen@uci.edu

Innate immune cells, such as monocytes, are among the first cells recruited to sites of infection and play a key role in host defense against *Toxoplasma gondii* infection. We have previously shown that *T. gondii*-infected primary human peripheral blood monocytes produce IL-1β through a Syk-PKC-δ-CARD9-MALT1-NF-κB signaling pathway, and IL-1β release requires the NLRP3 inflammasome and caspase-1 activity. To investigate a potential role of other caspases in IL-1β release, we conducted CRISPR/Cas-9 genome editing to knock out caspase-1, -4, -5, or -8 in THP-1 cells. Genetic ablation of caspase-1 or -8, but not caspase-4 or caspase-5, decreased IL-1β release during *T. gondii* infection. Furthermore, dual pharmacological inhibition of caspase-8 with IETD and RIPK1 with necrostatin-1 in primary human peripheral blood monocytes decreased IL-1β release without effecting cell viability or infection efficiency. Caspase-8 was not required for the production or cleavage of IL-1β but rather, caspase-8 inhibition led to the retention of mature IL-1β within the cells. Our data suggest that during type II *T. gondii* infection of human monocytes, caspase-8 functions in a novel gasdermin D-independent mechanism controlling IL-1β release from viable cells. This study expands on the molecular mechanisms of IL-1β release from human immune cells and on the inflammatory role of caspase-8 in host defense. Additional research from our laboratory has investigated the role of monocytes in innate immunity at the blood-brain barrier and in the central nervous system and the molecular cues guiding these cells to sites of *T. gondii* infection.

RT03-4 - **Cell invasion by Leishmania infective forms: unveiling hidden routes of infection and their possible impacts**

GOMES, T.D.C..
UFMG - MG - BRA
E-mail: tcgbio@yahoo.com.br

Parasites of the genus Leishmania, the causative agents of the different forms of Leishmaniasis, present two distinct evolutive forms: the extracellular promastigotes and the intracellular amastigotes. During the chronic phase of the disease in mammals, the amastigotes are mainly found inside macrophages. However, several other cell types, including non-phagocytic cells, have been found harboring intracellular amastigotes *in vivo*. Regarding their invasion processes, these parasites were long considered as passive players, relying only on the phagocytic abilities of phagocytes to invade host cells. However, the fact that amastigotes are also found within non-phagocytic cells shows that infection routes other than classic phagocytosis must occur. Therefore, we tackle to investigate the process of cell entry by both promastigotes and amastigotes of *Leishmania amazonensis* in non-phagocytic cells in order to define the cell biology basis of invasion. Our results show that both evolutive forms are able to actively induce cell entry in non-phagocytes where they live, replicate and persist within typical acidic compartments rich in lysosomal markers. Nonetheless, the route of cell invasion depends on the evolutive form of the parasite involved. Our data show that promastigotes can actively induce their own entry into these cells independently of host cell actin cytoskeleton activity, thus by a mechanism that is distinct from phagocytosis. Invasion involves subversion of host cell functions such as calcium signaling and recruitment and exocytosis of host cell lysosomes involved in plasma membrane repair, similarly to what was observed during *Trypanosoma cruzi* invasion, pointing to the conservation of this mechanism in these intracellular Trypanosomatids. On the other hand, cell entry by amastigotes in non-phagocytic cells is highly dependent on host cell cytoskeleton and involves an intense, but localized, re-arrangement of host cell F-actin at the entry site. Future studies about the impact of these new infection routes allowing these parasites to hide within non-phagocytic cells and on the role of these cell types in the course of infection may provide important information about the biology of these parasites and the diseases they cause. The presence of *Leishmania* spp. amastigotes within 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 drug resistance and late infection reactivation. ***Keywords*:** Leishmania spp; - Cell Invasion ;Parasite;Host Cell Interaction.

RT.04 – Next generation (not sequence!) of protozoologists

RT04-1 - Regulation of Transcription in *Leishmania* by Bromodomain Factor 5 and the CRKT complex

JONES, N.[1]; RUSSELL, C.[1]; GEOGHEGAN, V.[1]; MOORE, G.[1]; CARNIELLI, J.B.[1]; NEWLING, K.[1]; CALDERÓN, F.[2]; WILKINSON, T.[1]; MOTTRAM, J.C.M.J.C.[1].
1. UNIVERSITY OF YORK, GRB; 2. GSK, TRES CANTOS - ESP
E-mail: nathaniel.jones@york.ac.uk

Alongside other organisms in kinetoplastida, *Leishmania* have evolved an unusual genome architecture that requires RNA polymerase II transcribed genes to be expressed from polycistronic transcription units, with broad transcriptional start regions defined by histone variants and histone lysine acetylation. However, the way these chromatin marks are interpreted by the cell is not understood. Seven predicted bromodomain factors (BDF1-7), the reader modules for acetyl-lysine, were identified in the *Leishmania mexicana* genome. Cas9-driven gene deletions indicate that BDF1-5 are essential in promastigotes, whilst DiCre inducible gene deletion of the dual-bromodomain factor BDF5 identified it to be essential for both promastigotes and amastigotes. ChIP-seq assessment of BDF5s genomic distribution revealed it as highly enriched at transcriptional start sites. Using an optimised proximity proteomic and phosphoproteomic technique, XL-BioID, we defined the BDF5-proximal environment to be enriched for other bromodomain factors, histone acetyltransferase 2, and proteins essential for transcriptional activity and RNA processing. Inducible deletion of BDF5, led to a disruption of pol II transcriptional activity and global defects in gene expression. The proteins that form stable interactions with BDF5 likely represent a conserved regulator of kinetoplastid transcription (CRKT) complex, as orthologs are found in most other kinetoplastid species. Our results indicate the requirement of *Leishmania* to interpret histone acetylation marks for normal levels of gene expression and thus cellular viability. Furthermore, we have identified acetylated peptides capable of binding BDF5 bromodomains which have enabled the development of fluorescent polarisation assays to characterise binding specificity and to provide a platform for inhibitor screening assays, indicating that BDF5 might represent a potential drug target. ***Supported by:*** This work was supported by funding from GSK through the Pipeline Futures Group and a Fellowship from a Research Council United Kingdom Grand Challenges Research Funder under grant agreement 'A Global Network for Neglected Tropical Diseases' grant number MR/P027989/1. to Nathaniel Jones. This work was part-funded by the Wellcome Trust [ref: 204829] through the Centre for Future Health (CFH) at the University of York. ***Keywords*****:** Leishmania;epigenetics;bromodomains.

RT04-2 - *Giardia duodenalis* reshapes intestinal mucosal immunity to prevent tissue damage and attenuate *Toxoplasma gondii*-driven inflammation

SARDINHA-SILVA, A.[1]; COELHO, C.H.[2]; GAZZINELLI-GUIMARAES, P.H.[3]; STEWART, D.[1]; TJHIN, E.T.[1]; GREGG, B.[1]; FINK, M.[4]; SINGER, S.M.[4]; GRIGG, M.[1].
1. MOLECULAR PARASITOLOGY SECTION, LABORATORY OF PARASITIC DISEASES - NIAID, - *USA*; 2. , GEORGETOWN UNIVERSITY- *USA*; 3. 3HELMINTH IMMUNOLOGY SECTION, LABORATORY OF PARASITIC DISEASES, NIAID, NATIONAL INSTITUTES OF HEALTH, - *USA*; 4. DEPARTMENT OF BIOLOGY, GEORGETOWN UNIVERSITY, *USA* E-mail: aline.sardinhadasilva@nih.gov

Enteric infections that cause diarrheal disease are the second-leading cause of death in children worldwide. Recent epidemiological studies identified a decreased incidence and severity of life-threatening diarrhea in those children co-infected with the intestinal protozoan parasite *Giardia duodenalis.* To determine precisely how *Giardia* infection ameliorates tissue damage and reshapes mucosal immunity to confer a host protective effect, we show that *Giardia* induces a robust type-2 associated cytokine response, an antigen specific Th2 immune response within the lamina propria and causes a precipitous expansion of IL-10-producing CD4 T cells, that protects the host from tissue damage. To demonstrate that this "protist" can function as a potent anti-inflammatory agent, we induced a Th1-driven/IFN-gamma-mediated lethal ileitis by co-infecting *Giardia*-infected mice with *Toxoplasma gondii*, an intestinal protozoan parasite that causes a Crohn's disease-like enteritis. We found that the presence of *Giardia* significantly reduced *Toxoplasma*-mediated inflammation in the small intestine, by downregulating the frequency of $Tbet^{+}IFN\text{-}y^{+}Foxp3^{+}IL\text{-}10^{+}$ Th1 cells. Moreover, *Giardia* induced the expansion of IL-10 producing $ST2^{+}CD4+$ T cells expressing GATA-3, which downregulated *Toxoplasma*-driven IFN-y. Recombinant IL-10 injection only reduced the frequency of Th1 cells but was not sufficient to completely revert the histopathology induced during *Toxoplasma* infection, suggesting the importance of a cellular component. We are currently examining whether *Giardia* colonization can confer protection from other inflammatory driven processes associated with, for example, DSS-induced colitis.

RT04-3 - **Disruption of a single IP6K allele alters cell morphology and leads part of the *Trypanosoma cruzi* population to quiescence**

DA SILVA, M.S.[1]; ABUCHERY, B.E.[1]; PASSOS, A.D.O.[1]; MOTTA, M.C.M.[2]; CALDERANO, S.G.[3].
1. UNESP, BOTUCATU - SP - BRA; 2. INSTITUTE OF BIOPHYSICS CARLOS CHAGAS FILHO, UFRJ - BRA; 3. BUTANTAN INSTITUTE, SP BRA.
E-mail: marcelo.santos-silva@unesp.br

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 PP-IPs 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. However, our analyses suggests that these organisms lost orthologs for PP-IP5K in the evolutionary course, possibly during the transition from free-living to a parasitic lifestyle. Using the CRISPR/Cas9 approach and two rounds of 'sgRNA' transfection, we were able to disrupt the single and double alleles of IP6K in *Trypanosoma cruzi* (the causative agent of Chagas Disease), generating $IP6K^{-/+}$ and $IP6K^{-/-}$ lineages, respectively. IP6K inactivation causes several morphological effects in both lineages, such as rounding and wrinkling of the cell body, increased number of glycosomes, and mitochondrial enlargement. Notably, $IP6K^{-/-}$ lineage was unable to proliferate, and most *T. cruzi* cells died a few days after transfection, suggesting IP6K is essential to this organism. Curiously, $IP6K^{-/+}$ lineage showed a slight cell cycle arrest at G0/G1 phase. However, the arrested population showed no DNA damage. Then, after estimate the cell cycle phases length and the doubling-time, we developed a pioneering assay to measure quiescent cells based on negative EdU (5-Ethynyl-2′-deoxyuridine) labeling. The result of this assay suggests that the presence of IP6K is important to keep *T. cruzi* committed with the cell cycle. Together, our preliminary data suggests that the loss of IP6K has harmful consequences for *T. cruzi*, which points this kinase as a potential target for drug development, given that its identity relative to its human homolog is ~ 15%. Furthermore, these findings can contribute to a better understanding of the pyrophosphorylation performed by IP_7, an apparent non-enzymatic post-translational modification still little studied. **Supported by:** São Paulo Research Foundation (FAPESP)
Keywords: Inositol pyrophosphates;inositol heptakisphosphate;quiescence, Chagas disease, potential drug target.

RT04-4 - **Transcriptomic analysis revealed a conserved protein regulated by arginase activity in *Leishmania amazonensis***

AOKI, J.[1]; ZAMPIERI, R.A.[1]; HORÁCIO, E.C.A.[2]; NAHUM, L.A.[2]; FLOETER-WINTER, L.[1].
1. INSTITUTO DE BIOCIÊNCIAS DA *USP*- BRA; 2. FIOCRUZ, INSTITUTO RENÉ RACHOU - INSTITUTO DE CIÊNCIAS BIOLÓGICAS, UFMG, BRA
E-mail: juaoki@gmail.com

Arginase is an enzyme that uses L-arginine to produce urea and ornithine, being this last one a precursor of polyamines pathway. The importance of polyamines pathway in *Leishmania* is related to parasite replication and infectivity. Using RNA-seq technology to compare both promastigotes and axenic amastigotes of *L. amazonensis* arginase knockout and *L. amazonensis* wild type, we identified a differential transcriptomic profile and coordinate responses in the absence of arginase activity. The Top 5 list among the most differentially expressed genes revealed that the transcript of a hypothetical protein is increased 3.98-fold in parasites in the absence of arginase. The identification and characterization of hypothetical proteins is important not only for functional genomics, but also to improve the knowledge about signaling pathways, metabolism, stress response, drug resistance, as well as for the identification of new therapeutic targets. According to *in silico* analysis, this hypothetical protein is conserved among several *Leishmania* species, presents a signal peptide in the N-terminal region, no conserved domains, no transmembrane domain and an intrinsically disordered region. Despite the lack of a stable 3D structure, intrinsically disordered proteins comprise a large and functionally important class with highly flexible conformation and role in many biological processes, such as regulation of transcription, translational and signal transduction. The characterization of this protein comprises the generation of a null mutant that can provide important insights on how *Leishmania* is able to modulate the mechanisms of gene expression regulation to allow the parasite survival. **Supported by:** FAPESP (#2021/04422-3 and #2018/23512-0) and CAPES (#88887.502799/2020-00). **Keywords:** RNA-seq;differentially expressed genes;disordered protein.

HP-01 - **Gasdermin-D activation in response to *Leishmania* infection induce a transient cell permeabilization to promote NLRP3 activation and host resistance to infection**

DE SÁ, K.S.G.[1]; AMARAL, L.A.[2]; RODRIGUES, T.S.[2]; ISHIMOTO, A.Y.[2]; DE ANDRADE, W.A.C.[2]; DE ALMEIDA, L.[2]; CASTRO, F.F.[2]; BATAH, S.S.[2]; PASTORELLO, M.T.[2]; FABRO, A.T.[2]; ZAMBONI, D.S.[2].
1. DEPARTAMENTO DE BIOLOGIA CELULAR E MOLECULAR E BIOAGENTES PATOGÊNICOS, FMRP, BRA; 2. USP, RIBEIRÃO PRETO - SP - BRA.
E-mail: keyla-sa@hotmail.com

Leishmania is an obligate intracellular parasite that causes Leishmaniasis, a disease that affects millions of people worldwide. *Leishmania* evades immune response by inhibiting specific processes on parasite-containing immune cells, yet the NLRP3 inflammasome activation is key for disease outcome. The molecular mechanisms upstream of the inflammasome activation are still unclear and there is no evident host cell death in *Leishmania*-infected cells. Here, we investigated the participation of Gasdermin-D (GSDMD, a pore-forming effector protein associated with pyroptosis) during *Leishmania* infection in macrophages and in vivo. We demonstrated that despite the absence of pyroptosis, GSDMD is active at the early stages of *L. amazonensis* infection in macrophages, allowing a transient cell permeabilization and potassium efflux, promoting NLRP3 inflammasome activation. *Gsdmd*$^{-/-}$ macrophages exhibit less ASC puncta formation and IL-1β production in response to infection, suggesting that the transient GSDMD-mediated permeabilization contributes for NLRP3 inflammasome activation. Mouse and macrophages deficient in GSDMD were highly susceptible to infection by several *Leishmania* species, including *L. amazonensis, L. major, L. braziliensis* and *L. mexicana*, confirming a key role of Gasdermin-D for inflammasome-mediated host resistance to infection. Finally, ASC/NLRP3 puncta and cleaved Gasdermin-D were present in skin biopsies of leishmaniasis patients, supporting the role of these molecules during active disease in humans. Altogether, our findings reveal that *Leishmania* infection triggers a transient activation of GSDMD and this molecule is critical for inflammasome activation and immunity in Leishmaniasis.
Supported by:FAPESP 2018/16777-8 **Keywords:**Leishmania;Inflammasome;GSDMD.

HP-02 - **Technological development of a new vaccine candidate against canine visceral leishmaniasis caused by *Leishmania infantum*.**

MELO JUNIOR, O.A.D.O.[1]; DOS SANTOS, J.S.[1]; GONDIM, F.C.[1]; MACIEL, D.O.[1]; BORSODI, M.P.G.[1]; DE FARIAS, V.P.[2]; LIMA, E.M.[3]; MOREIRA, O.D.C.[4]; SOUSA-BATISTA, A.D.J.[1]; GUEDES, H.L.D.M.[1]; BERGMANN, B.R.[1].
1. UFRJ- RJ - BRA; 2. UNIVERSIDADE FEDERAL DO RIO DE JANIERO, RJ- BRA; 3. UNIVERSIDADE FEDERAL DE GOIAS, GOIANIA - GO - BRA; 4. INSTITUTO OSWALDO CRUZ, RJ- BRA.
E-mail: otoni@biof.ufrj.br

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 (LaAg RA-LipPEG) 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 LaAg RA-LipPEG in preventing parasite growth in the spleen, liver and bone marrow after infection. Also, a clinical trial with 30 L. infantum-free mongrel dogs, non-reactive by SNAP, ELISA and PCR, is under way. Dogs received two or three intranasal doses of LaAg RA-LipPEG at 15- day intervals, or 3 subcutaneous doses of the marketed Leish-Tec ® as controls. LaAg RA-LipPEG 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. Dogs were challenged with L. infantum 3 months after vaccination and tested for PCR 2 months after infection. Overall, LaAg RA-LipPEG is a promising candidate for the prevention of canine visceral leishmaniasis caused by L. infantum. **Supported by:**Global NTD Network **Keywords:**Canine Visceral Leishmaniasis ;Vaccine;Intranasal.

HP-03 - **TNFR1, the maestro of inflammatory response to infection with *Leishmania amazonensis***

VAZ, L.G.; DINIZ, C.; DE ANDRADE, A.C.; NOLASCO, A.E.; AMARAL, F.; VIEIRA, L.Q..
UFMG - MG - BRA.
E-mail: leoc_bio@yahoo.com.br

TNF is pleiotropic cytokine involved in inflammation, host defense, tissue degeneration and tissue regeneration, among other functions. TNF acts through two cognate receptors. Signaling throughTNFR1 is paramount against intracellular parasites, including *Leishmania*. We investigated the impact of TNFR1 on the infection by *L. amazonensis* using wild-type (WT) and TNFR1 knockout (TNFR1ko) mice. Although expression of TNFR1 by WT mice is not enough to eliminate the parasite, this receptor mediates control of parasite replication and inflammatory response. At nine weeks, lesions were larger in TNFR1ko mice, and at 12 weeks both lesions and parasite number were larger in TNFR1 ko mice. WT mice presented more $F4/80^{+}MHCII^{+}$ cells (P3) than TNFR1ko mice at 9 and 12 weeks of infection. At 9 weeks WT had more $iNOS^{+}$ and arginase 1 (Arg1) double positive $iNOS^{+}Arg1^{-}$ in P3 cells than TNFR1ko mice. At 12 weeks of infection, $iNOS^{-}Arg1^{+}$ P3 cells were higher in WT than in TNFR1ko mice. We could not detect differences in the number or percentage of infected myeloid cells or in the presence of NO in these cells *in vivo*. However, BMDM derived from TNFR1ko mice were more susceptible to infection with *L. amazonensis.* Moreover, these cells produced less NO when activated with IFN-γ and infected with *L. amazonensis.* We also found larger number of T lymphocytes in TNFR1 ko mice. Furthermore, most of the few lymphocytes found in WT mice at 12 weeks of infection were regulatory T cells. At this time point we also found higher levels of IL-10 in lesions of WT mice. In summary, at the last time point of infection we investigated, WT mice presented a regulatory response characterized by macrophages $iNOS^{-}$ $Arg1^{+}$ (M2), Tregs at the site of infection and production of IL-10. We conclude that for successful clearance and wound healing during leishmaniasis, different phenotypes of macrophages need to appear coordinately in the appropriate time. TNF, through TNFR1, seems to act as this conductor. **Supported by:**CAPES, CNPq, FAPEMIG **Keywords:**TNFR1;Leishmania;macrophage polarization.

HP-05 - **GLYCOCONJUGATES (LPG/GIPLS) FROM DERMOTROPIC AMAZONIAN *LEISHMANIA* SPECIES DISPLAYS INTERSPECIES VARIATIONS IN THEIR BIOCHEMICAL AND FUNCTIONAL PROPERTIES IN C57BL/6 MACROPHAGES**

SOARES, R.P.P.[1]; RÊGO, F.D.[1]; LAURENTI, M.D.[2].
1. INSTITUTO RENÉ RACHOU/FIOCRUZ, BH- MG - BRA; 2. FACULDADE DE MEDICINA DA USP/FMUSP, SÃO PAULO - SP - BRA.
E-mail: rsoares@cpqrr.fiocruz.br

Lipophosphoglycans (LPGs) and Glycoinositolphospholipids (GIPLs) are *Leishmania* glycoconjugates involved in host-parasite interaction in the vertebrate host. Information on the glycobiology of dermotropic Amazonian *Leishmania* species is scarce. For this reason, the objectives of this project were to report the biochemical and functions properties of LPGs and GIPLs. The species included: *Leishmania braziliensis*, *L. guyanensis*, *L. shawi*, *L. lansoni*, *L. lindembergui* and *L. naiffi*. After extraction and purification of LPGs and GIPLs, the preliminary biochemical structures were obtained using fluorophore-assisted carbohydrate electrophoresis. Interesting polymorphisms were observed in the LPGs and GIPLs of the six species. Most of the LPGs were devoid of sidechains (type I) showing the typical Gal-Man-PO_4 repeat units. Those included *L. shawi, L. lainsoni*, *L. lindembergui* and *L. naiffi*. LPGs of *L. braziliensis* and *L. guyanensis* showed galactose and glucose sidechains, respectively. GIPLs from dermotropic *Viannia* species also had variations in their monosaccharide content. GIPLs from *L. guyanensis* and *L. naiffi* possesses galactose and mannose (Type II/hybrid), whereas for the other species, galactose was the main sugar (Type I). Those glycoconjugates were assayed in mouse peritoneal macrophages and respective TLR2 -/- and TLR4 -/- knock-outs for NO and cytokine/chemokine production. Regardelless the species, NO and cytokine/chemokine production were primarily via TLR4/TLR2. A higher pro-inflammatory activity was detected in *L. lainsoni* (type I LPG). We did not establish any correlation between biochemical structure and functional activity. In conclusion, there are several biochemical polymorphisms in the LPGs and GIPLs of Amazonian dermotropic *Viannia* species. Those glycoconjugates triggered variable innate immune responses in macrophages and may contribute to the spectrum of clinical manifestations in dermotropic *Viannia* species. **Supported by:**FAPESP (2021/01243-0); CNPq (302972/2019-6); FAPEMIG PPM-XII (00202-18) **Keywords:** LIPOPHOSPHOGLYCAN; GLYCOINOISTOLPHOSPHOLIPIDS; INNATE IMMUNITY.

HP-06 - **Congenital Toxoplasma gondii infection affects retinal proliferation and differentiation in mice.**

DE CAMPOS, V.S.[1]; DA COSTA, K.C.[1]; ADESSE, D.P.[2]. 1. UNIVERSIDADE FEDERAL FLUMINENSE, NITERÓI - RJ - BRA; 2. FIOCRUZ, RJ- BRA.
E-mail: vvncampos@gmail.com

Toxoplasmosis affects one third of the world population and has the protozoan *Toxoplasma gondii* as its etiological agent. Congenital infection (CI) can cause severe damage to the fetus, such as abortions, intracranial calcification, hydrocephalus and retinochoroiditis. The severity of the impairment depends on the gestational period in which infection occurs. Even so, there are few data that clearly demonstrate the occurrence of alterations at the cellular level during the stages of retinal development, after congenital infection. The present work aims to investigate the impact of CI by *T. gondii* on the retina of mice. We proposed a model for CI, in which pregnant females of the C57bl6 strain are separated into two groups, control and infected, and the offspring are analyzed at embryonic day (E) 18 and E20. At E10, pregnant females are infected intragastrically with 2 cysts of the Me49 strain of *T. gondii,* while controls received saline solution. At E18 and E20, the infected pups had significantly smaller body size and weight than the controls, indicating that embryonic development was affected. A significant increase in the number of Ki67-positive cells (marker of proliferating cells) in the neuroblastic layer (NBL) and an augment in the number of cells in mitosis in the apical region of the NBL was detected in the retina of the infected mice compared to the control. In agreement, cell cycle proteins, such as cyclin D3, Cdk6 and pChK2, were also significantly altered in infected retinas. Interestingly, the immunohistochemical analysis showed a significant increase in the population of β-III-tubulin-positive cells, one of the earliest markers of neuronal differentiation. Together, the data suggest that CI alter cell cycle, increasing proliferation but inducing the arrest of these cells at G2/M phase. It is possible that these alterations influence the differentiation, anticipating/increasing neuronal maturation, and therefore leading to abnormal retinal formation. **Supported by:**INOVA no. 3231984391; CNPq 401772/2015-2 - 444478/2014-0;INCT/INNT 465346/2014-6 for KC ,FAPERJ no. E-26/010-001199/2015; Projetos Temáticos no. E26/010.101037/201; Sediadas grant no. E-26/010.001493/2019 for KC). **Keywords:**Congenital toxoplasmosis;Retina;Development.

HP-09 - **Modifications in lipid metabolism of swiss mice infected with *Toxoplasma gondii*.**

ANDRADE, S.V.D.; CABRAL, S.S.; KLUCK, G.E.G.; MARTINS-DUARTE, É.D.S.; DE SOUZA, W.; VOMMARO, R.C.; ATELLA, G..
UFRJ- RJ - BRA.
E-mail: savadean22@hotmail.com

Toxoplasmosis is a neglected tropical disease of which Brazil stands out as the third country with highest prevalence of IgG against this parasite. The disease, caused by an intracellular protozoan of the genus Toxoplasma, affects the felids and several other species, as humans. *Toxoplasma gondii* does not have complete degradation and synthesis lipid pathways and, thus, it depends on hosts for its development. Therefore, the aim of this work is to characterize the liver lipid metabolism of mice infected by *Toxoplasma gondii***.** For this, 3 groups of Swiss mice, male and female, were submitted to an infection time course of 3,4,7 weeks or 4 months, at two different parasite loads, 50 and 250 parasites (CTR, n=32; INF 50, n=28; INF 250, n=27). After the infections, livers were submitted to proteins, cholesterol, glucose and triacylglycerols (TAG) dosage using enzymatic colorimetric method (DOLES). Also, lipids were extracted and submitted to thin layer chromatography (TLC) to assess whether the infection would alter the lipid metabolism. From the results obtained, it was observed a significant decrease in the amount of proteins in 7-week-old females and males, a significant reduction in TAG concentration in 3-week-old females and in 4-month-old females; a significant glucose decrease in females and 3-week-old males and a cholesterol decrease in 7-week-old females. About the lipids assess, it was observed a significant increase in TAG and phospholipids and, also, a decrease of monoacylglycerol (MAG) in 3-week-old females; a decrease of TAG in 7-week-old females; a decrease in esterified cholesterol, TAG, 1,3-diacylglycerol and MAG in 4-month-old females. Thus, *Toxoplasma gondii* modulates lipid metabolism in the liver of Swiss mice. These alterations may be involved as an attempt to acquire these macromolecules for its own benefit, in order to complete their life cycle. **Supported by:**FAPERJ, CNPq, INCT **Keywords:** Metabolism; Lipids; Toxoplasma gondii.

HP-10 - **Modulation of the Host Nuclear Compartment by *Trypanosoma cruz*i Uncovers Effects on Host Transcription and Splicing Machinery**

DE CASTRO, C.G.[1]; CASTRO, F.F.[2]; CÓRDOVA, R.A.G.[3]; DA FONSECA, C.K.[3]; GOMES, M.D.[3]; ISHIKAWA-ANKERHOLD, H.C.[4]; BAQUI, M.M.A.[3].
1. INSTITUTO BUTANTAN, SÃO PAULO - SP - BRA; 2. USP - SP - BRA; 3. FMRP-USP, RIBEIRÃO PRETO - SP - BRA; 4. UNIVERSIDADE DE MUNIQUE, MUNICH - ALEMANHA.
E-mail: camilagachet@gmail.com

Host manipulation is a common strategy for invading pathogens. *Trypanosoma cruzi*, the causative agent of Chagas Disease, lives intracellularly within host cells. During infection, parasite-associated modifications occur to the host cell metabolism and morphology. However, little is known about the effect of *T. cruzi* infection on the host cell nucleus and nuclear functionality. The methodology used in LLC-MK2 , HeLa and THP-1 cells infected with *T. cruzi* compared to uninfected cells (control) was based on analysis of images obtained in a confocal and electron microscope, of gene and protein expression, and the use of a mini reporter gene for check splicing *in vivo*. The results showed that the parasite migrates close to the nucleus causing deformation in the nuclear envelope, and altering the chromatin conformation during the infection. Also, we showed that *T. cruzi* can modulate host transcription and splicing machinery in non-professional phagocytic cells during infection. We found that *T. cruzi* regulates host RNA polymerase II (RNAPII) in a time-dependent manner, resulting in a drastic decrease in RNAPII activity. Furthermore, host cell ribonucleoproteins associated with mRNA transcription (hnRNPA1 and AB2) are downregulated concurrently. We reasoned that *T. cruzi* may hijack the host U2AF35 auxiliary factor, a key regulator for RNA processing, as a strategy to affect the splicing machinery activities directly. In support of our hypothesis, we carried out *in vivo* splicing assays using an adenovirus E1A pre-mRNA splicing reporter, showing that intracellular *T. cruzi* directly modulates the host cells by appropriating U2AF35. For the first time, our published results provide evidence of a complex and intimate molecular relationship between *T. cruzi* and the host cell nucleus during infection. **Supported by:**FAPESP 2010/19547-1; 2018/03677-5; CAPES finance code 001; FAEPA-FMRP-USP **Keywords:**host-pathogen interaction;trypanosomatid;nucleus.

HP-11 - **Mechanisms of FAZ assembly in *Trypanosoma cruzi***

MELO, N.S.; ALCANTARA, C.D.L.; VIDAL, J.C.; ROCHA, G.M.; DE SOUZA, W..
UFRJ, RJ- BRA.
E-mail: normandasouzamelo@gmail.com

T. cruzi has a single flagellum responsible for motility. The flagellum/cell body connection is established via the flagellar attachment zone (FAZ), an adherent network composed of a set of fibers, filaments, and junctional complexes that starts from the flagellum domain and is anchored to the cell cytoskeleton and remains attached along the body, except the distal portion of the flagellum. Assembly dynamics of FAZ are poorly characterized in *T. cruzi*, although in other trypanosomatids it is a key regulator of cellular processes. Despite the classic phenotype and depletion of TcGP72 well established little is known about interaction with other proteins and its structural features during the life cycle of *T.cruzi*. TcGP72 protein is a homolog to FLA1, 2 e 3 of *T. brucei*. In *T. brucei* it was described that TbFLA1 protein interacts with FLA1 Binding Protein (TbFLA1BP) promoting the membrane adhesion between the flagellum/cell body. This TbFLA1/TbFLA1BP association favors the assembly of FAZ and flagellum and is responsible for the regulation of morphogenesis. In this study we analyze the role of GP72 and FLA1BP proteins during the life cycle of *T. cruzi*, evaluating tagged parasites and knockout for both proteins using CRISPR/Cas9 system. We report that C-terminal is required for correct targeting of the TcGP72 and mutants labeled on the C-terminal showed flagellum detachment as well as a knockout. TcFLA1BP protein exhibits localization along the FAZ in the cell body in all phases of the cell cycle. Consistent with previous studies we observed that the TcGP72 -/- showed flagellum detachment from the cell body in all *T. cruzi* forms, in addition, to presenting drastic morphological changes in organelle positioning during metacyclogenesis and altered flagellum/cell length. TcFLA-1BP -/- also showed morphological changes, flagellum partially detached flagellum and ratio flagellum/cell size diminished. Both knockouts exhibited inhibition of growth, cytokinesis, and infection. **Supported by:**FAPERJ **Keywords:**flagellar attachment zone;FAZ protein;GP72/FLA1 Binding Protein.

HP-13 - **Physical exercise protects mice from muscular and neural pathology after *Toxoplasma gondii* infection**

VIEIRA, P.D.C.[1]; DE CASTRO, L.L.[2]; ESTRELA, L.P.[1]; RODRIGUES, K.L.[1]; PEREIRA, E.N.G.[1]; DALIRY, A.[1]; BARBOSA, H.S.[1]; ADESSE, D.P.[1].
1. FIOCRUZ, RJ- BRA; 2. UFRJ, RJ- BRA.
E-mail: paloma.vieira.rj@gmail.com

Toxoplasmosis has a worldwide distribution and is caused by the intracellular parasite, *Toxoplasma gondii*. Acquired toxoplasmosis can be lead to myositis, polymyositis and cardiac compromising. Although etiological treatments are available, none of them can fully prevent effects of the disease. Physical exercise (PE) has been described as a non-invasive treatment for many diseases such as diabetes, obesity, and has preventive action for others such as anxiety and cancer. We evaluated if PE could prevent the effects of acquired Toxoplasmosis. Swiss Webster male mice were trained for 8 weeks in an aerobic wheel for 5 days/week in 30-min sessions at 60% speed of exhaustion capacity. O_2 consumption (VO_2), maximum speed and time of exhaustion were evaluated during the protocol. After training period, mice were infected intragastrically with 10 cysts of ME49 *T. gondii* strain. Every 2 days after infection, grip strength was evaluated, and 10 days post infection animals were evaluated for aerobic performance and microcirculatory parameters of muscle and brain. As expected, PE increased maximum speed and time until exhaustion. *T. gondii* infection decreased the maximum speed (VO_2 at rest and maximum VO_2 during the exercise) in sedentary animals. Grip strength peak was also increased with exercise, and although it did not change with infection, the test time was reduced in sedentary-infected mice. Tibialis anterior muscle (TA) showed no weight change, but soleus muscle weight increased with training but not after infection. *T. gondii* infection decreased brown, but not epididymal and retroperitoneal white adipose tissue. Laser speckle imaging showed an extensive reduction on TA and brain blood flow in sedentary infected mice, which was prevented by PE. Rolling and adherent leukocytes was also drastically increased in sedentary infected mice, but not in exercised ones. Our results indicate that PE is a powerful tool for preventing muscle and cerebral damages of toxoplasmosis. **Supported by:**INOVA Fiocruz, IOC, CNPq, Edital PAPES VII, Faperj **Keywords:**Toxoplasmosis;Physical exercise;Muscle and cerebral damage.

HP-15 - **PROFILING OF ANGIOGENIC FACTORS RELEASED BY TOXOPLASMA GONDII CYST-BEARING CORTICAL NEURONS**

DE CASTRO, L.L.[1]; DINIZ, L.[2]; SCHARFSTEIN, J.[1]; ADESSE, D.P.[3].
1. INSTIUTO DE BIOFÍSICA CARLOS CHAGAS FILHO | IBBCF - UFRJ, RJ- BRA; 2. INSTITUTO DE CIÊNCIAS BIOMÉDICAS | ICB - UFRJ, BRA; 3. INSTITUTO OSWALDO CRUZ | IOC - FIOCRUZ, RJ- BRA
E-mail: leonardoleal@biof.ufrj.br

Central Nervous System is a major target of *Toxoplasma gondii* infection *in vivo* and tissue cysts are found primarily in neurons. Recent evidence indicates that acquired toxoplasmosis in mice can lead to neuro-inflammation and -degeneration as well as behavioral abnormalities. We have shown that *T. gondii* reduces cerebral blood flow and leads to a microvascular rarefaction, with increased Blood-Brain Barrier (BBB) permeability. Because *T. gondii* resides predominantly in neurons and the cellular interactions of the BBB with other neural cells are relevant for the maintenance of BBB integrity, we evaluated the pro- and anti-angiogenic factors released by *T. gondii* cyst-bearing neurons. Primary cultures of Swiss Webster mouse embryo cortical neurons were infected with tachyzoites of ME49 strain. We confirmed that our cultures were 100% neuron-enriched, as showed by β-III-tubulin and neurofilament immunostaining. Moreover, tissue cysts were found, as shown by CST-1 and DBA staining. At 7 dpi, culture supernatants were submitted to Proteome Profiler Angiogenesis Array Kit and we verified that VEGF, Fractalkine (CX3CL1), SDF-1 (CXCL12), NOV (CCN3), PDGF-AA and MMP-3 analytes were decreased in infected cultures. In order to further validate our findings in a relevant animal model of acquired toxoplasmosis, we analyzed the expression of genes of interest in the cortices of SW mice infected with *T. gondii* for 10 and 40 days, when neuroinflammation and BBB damage occur. ZO-1, a tight junction adaptor protein, was significantly increased in infected brains compared to controls, which corroborates our findings of microvascular dysfunction induced by infection. Fractalkine 3, a neuron-derived chemokine that signals to CX3CR1 in microglia, was reduced at 10 and 40 dpi by 0.71- and 0.68-fold, respectively. Our results point to a possible mechanism by which latent toxoplasmosis disturbs cerebral microvasculature, thus leading to neurological dysfunction. **Supported by:** Fiocruz, Programa INOVA Fiocruz, FAPERJ **Keywords:** cortical neurons; angiogenesis; toxoplasma gondii.

HP-16 - **CHARACTERIZATION OF REGULATION MECHANISMS INVOLVED IN *LLCHIT1 EXPRESSION*, A MIDGUT-SPECIFIC CHITINASE OF *LUTZOMYIA LONGIPALPIS*, THE MAIN VECTOR OF AMERICAN VISCERAL LEISHMANIASIS.**

RIBEIRO, A.C.P.S.[1]; NEGREIRA, G.O.L.[2]; TRAUB-CSEKO, Y.M.[1]; TEMPONE, A.J.[1].
1. FIOCRUZ, RJ- BRA; 2. INSTITUTE OF TROPICAL MEDICINE ANTWERP, ANTWERPEN - BEL
E-mail: ac_arolina@outlook.com

Our group identified a midgut-specific chitinase, named *LIchit1*, in *Lutzomyia longipalpis*, the main vector of American Visceral Leishmaniasis. *LIchit1* expression is induced upon blood feeding and the level of *LIchit1* mRNA appears to peak at 72h PBM, when occurs the degradation of the peritrophic matrix (PM), an important event in the establishment of *Leishmania* infection in the vector. Aiming to understand the mechanisms involved in this gene regulation, we previously started the characterization of its promoter. The *LIChit1* promoter sequencing analysis revealed the presence of putative transcriptional factors binding sites including GATA, Ecdysone receptor (ECR), and Ecdysone-induced proteins (EIP). Through luciferase assays, we observed that, in this promoter, the exonuclease deletion of GATA factor binding sites interrupts the reporter induction. Furthermore, a fragment of this promoter encompassing the region between -1200 e +68 (P1268) containing a corresponding sequence to the ECR and binding sites to EIP strongly induced the luciferase expression upon ecdysone stimulus. In this way, the focus of the present work was to characterize the role of GATA and ecdysone on *LIChit1* expression. In *L. longipalpis* LL-5 embryonic cells we silenced the GATA factor and, through qPCR, we observed a decrease in *LIChit1* expression. Furthermore, incubating these cells with ecdysone we observed an increase in *LIChit1* expression. Considering that the blood meal may raise the production of both ecdysone and GATA through the TOR pathway, we evaluated the role of the mTOR kinase on *LIChit1* expression. Although GATA and ecdysone act like transcriptional activators of *LIChit1,* mTOR knockdown did not affect *LIChit1* expression. Thereby, the present work characterizes a sandfly promoter that, due to tissue and stimulus-specific expression becomes a possible tool for the development of transgenic sandflies refractory to *Leishmania* infection. **Supported by:**FAPERJ. 26/200.578/2020 **Keywords:**Lutzomyia longipalpis; Chitinase gene promoter; gene regulation.

HP-17 - **High-dimensional flow cytometry to evaluate CD4⁺ T cell heterogeneity during Chagas cardiomyopathy .**

ALMEIDA, G.G.[1]; RIMKUTE, I.[2]; DO VALE, I.N.P.C.[1]; LIECHTI, T.[2]; HENRIQUES, P.M.[1]; ROFFE, E.D.S.[2]; DE ARAUJO, F.F.[1]; ROCHA, M.O.D.C.[3]; SANTOS, S.M.E.[3]; MARTINS-FILHO, O.A.[1]; JANKOVIC, D.[2]; SHER, A.[2]; DE CARVALHO, A.T.[1]; ROEDERER, M.[2]; ANTONELLI, L.R.D.V.[1].
1. INSTITUTO RENÉ RACHOU, MG - BRA; 2. NIH, WASHINGTON - USA; 3. UFMG - BRA
E-mail: vet.greg@gmail.com

Chagas' disease is a neglected tropical disease caused by the infection of *Trypanosoma cruzi* protozoa. Chronic chagasic cardiomyopathy (CCC) is a result of a persistent myocarditis during Chagas' disease, leading to heart failure and death in several cases. Although much of the immune response during Chagas' disease is known to rely on cytotoxic T cells, the role of $CD4^+$ T cells is less understood. Also, a comprehensive analysis of activation markers on $CD4^+$ T cells during different clinical forms was not performed so far. To explore a broad phenotyping of $CD4^+$ T cell in different stages of Chagas' disease, we employed a high-dimensional flow cytometry panel combining 27 markers. Results were analyzed by both supervised and unsupervised strategies. Unsupervised strategy revealed several phenotypes of $CD4^+$ T cells that are differentially represented in the different stages of the disease. $CD69^+CD4^+$ T cells are expanded during Chagas' disease in all memory compartments and among naïve T cells, although decreased in mild CCC compared to both patients without cardiac disease and established CCC. In addition, regulatory T cells expressing CD39 display a lower frequency in mild CCC compared to controls. The frequency of $CD4^+$ T cells co-expressing granzyme B and perforin is increased in chagasic patients compared to controls and is more pronounced among patients with mild CCC compared to controls. Patients with mild CCC produce cytokines upon antigen stimulation and display higher frequencies of effector memory $CD4^+$ T cells with multifunctional phenotype. Altogether, our results showed an imbalance of proinflammatory and regulatory responses of $CD4^+$ T cells in the establishment of mild Chagas cardiomyopathy. Our results showed the heterogeneity of the phenotypes associated with disease worsening, providing insights on the pathology of the disease and potential markers to guide clinical decisions. **Supported by:**INCTV, Fiocruz, NIH **Keywords:**Chagas' disease;T cell;immunoresponse.

HP-18 - **ROLE OF P2X7 PURINERGIC MOLECULE IN RESPONSE TO *Trypanosoma cruzi* IN THE ACUTE PHASE OF CARDIAC INFECTION.**

XAVIER JUNIOR, J.T.[1]; SANTOS, G.D.A.[2]; DO NASCIMENTO, R.S.[2]; FEVEREIRO, M.R.[2]; DE SÁ, K.S.G.[1]; ZAMBONI, D.S.[1]; DE CHAVES, M.L.M.B.[2]; LIMA, M.R.D.I.[2]; MOSIG, J.M.A.[2].
1. USP, RP - SP - BRA; 2. USP - SP - BRA
E-mail: txjoaquim@outlook.com

Chagas disease, caused by *Trypanosoma cruzi*, is an important cause of acute and chronic cardiomyopathy worldwide. *T. cruzi* is capable of infecting and replicating in several cell types, which can lead to cell death by necrosis, releasing danger signals such as ATP. The extracellular ATP can be recognized by the P2X7 ion channel, thus being able to act in cell activation. However, overstimulation of this receptor can lead to cell death by pyroptosis or necrosis. Considering that, in the heart of *T.cruzi*-infected C57BL/6 mice we observed an increase in the number of cells expressing P2X7, as well as in the level of *P2rx7* transcripts. In this work we evaluated the role of P2X7 in the heart during an acute phase of infection by *T. cruzi*. *P2rx7* $^{-/-}$ infected mice showed no differences in body weight, blood parasitemia, heart weight and cellular infiltrate in the cardiac tissue when compared to C57BL/6 equally infected mice. However, an increase in *T.c. 18s* satellite DNA of *T. cruzi*, *IL-10* and *Arg1* mRNA transcripts, as well as a decrease in *IL-6, Nos2* and *IFN-γ* transcripts, was observed in the heart of the infected *P2rx7* $^{-/-}$ mice. Furthermore, in the protein analysis, the heart of *T. cruzi*-infected *P2rx7* $^{-/-}$ mice presented a reduction in the levels of IFN-γ and IL-1β and an increase in IL-10 and IL-12p70 when compared to C57BL/6 equally infected, indicating that in the cardiac tissue of *P2rx7* $^{-/-}$ mice, the effector cells of the immune system, such as macrophages, monocytes and lymphocytes, are less responsive to *T. cruzi* infection, maybe by the less signaling via NLRP3 inflammasome. However, no significant differences were observed in lymphocyte activation or macrophage polarization to M1 and M2. The data obtained suggest that the P2X7 molecule plays a role in macrophage activation, and its absence may culminate in the possible worsening of the disease. **Supported by:**FAPESP 2018/25984-7 **Keywords:**P2X7;Trypanosoma cruzi;Macrophages.

PV-01 - **Identification and testing of candidates for the development of leishmaniasis Transmission Blocking Vaccines**

LEMOS-SILVA, T.[1]; COELHO, F.S.[1]; TELLERIA, E.L.[1]; TEMPONE, A.J.[1]; SACKS, D.L.[2]; TRAUB-CSEKO, Y.M.[1].
1. IOC, FIOCRUZ, LABORATÓRIO DE BIOLOGIA MOLECULAR DE PARASITAS E VETORES, RJ-BRA; 2. NIH, DEPARTMENT OF LABORATORY MEDICINE, USA
E-mail: ytraub@ioc.fiocruz.br

There has been a significant increase in the number of human cases of visceral leishmaniasis in recent years, which in Brazil is caused by *Leishmania infantum chagasi*, transmitted by *Lutzomyia longipalpi.* There is a demand for new approaches in the control of leishmaniasis due to limitations in the use of traditional methods, such as insecticide resistance, costs, etc. Among these alternatives is the use of transmission blocking vaccines (VBTs). The targets of VBTs are pathogen or insect proteins responsible for establishing the pathogen in the vector. We are working with several promising candidates in the parasite, both testing insect infection with mutant parasites (either obtained through collaborations or by creating mutants through CRISPR) and performing artificial infections in the presence of antibodies against proteins of interest. Among the targets with promising results are genes involved in sugar and amino acid metabolism which are upregulated at times of high parasite proliferation inside the insect. As vector targets, we are investing in the identification of molecules involved in vectorial capacity. We are currently performing experiments where these target proteins of the vector or parasite are being inoculated into mice and/or hamsters, which will then be infected with *Leishmania* and exposed to *L. longipalpis* bites. Levels of vector infection are being determined in relation to insects fed on mock-immunized control animals.
-TLS is developing part of this work at the NIH during a Graduate Sandwich Fellowship.
-ELT is presently at the Charles University, Czech Republic. **Supported by:** INCTEM, IOC-Fiocruz, INOVA-Fiocruz, FAPERJ **Keywords:**Leishmaniasis;transmission blocking vaccines;vector-parasite interaction.

PV-02 - **Glucose Metabolism is Essential to Support Heme-Induced Epimastigotes Proliferation of Trypanosoma cruzi**

VIEIRA, C.S.D.[1]; AGUIAR, R.P.[2]; NOGUEIRA, N.P.D.A.[1]; DOS SANTOS JUNIOR, G.C.[1]; PAES, M.C.[1].
1. UERJ, RJ- BRA; 2. UFRJ, RJ - BRA.
E-mail: carolina.sd.vieira@gmail.com

Trypanosoma cruzi is the causative agent of Chagas disease, a neglected illness transmitted by triatomine insects during its blood meal. Heme, an abundant product of blood digestion, is a physiological oxidant molecule that triggers epimastigotes proliferation and transcriptionally regulates the expression of genes related to energy metabolism, mostly upregulating genes involved to glycolysis and aerobic fermentation process in epimastigotes. Once these metabolic pathways are essential to energy demand in this proliferative stage, this work aimed to investigate the glucose metabolism of epimastigotes cultured with heme through metabolomic and bioenergetic approaches. In response to heme, *T. cruzi* epimastigotes enhanced the D-glucose consumption after 24h and over the days, producing and secreting to its culture medium increased succinate levels, the main product of succinic fermentation of *T. cruzi*. High levels of succinate in supernatant promoted acidification of extracellular pH, being this effect inhibited by addition of glycolysis inhibitor, 2-deoxy-D-glucose (2-DG). Also, was shown that D-glucose supplementation increased epimastigotes proliferation exclusively in the presence of heme, and glycolysis inhibition impaired significantly the heme-induced proliferation. Regarding the mitochondrial physiology, heme only increased epimastigotes electron transport system-related O_2 consumption rate (OCR) compared to control. But, immediately after D-glucose addition, this OCR was impaired, similar a Crabtree effect (the glucose-mediated inhibition of mitochondrial respiration). Taken together, our data substantiate the idea previously pointed by transcriptional analysis, that heme signaling modulate the energy metabolism of *T. cruzi* epimastigotes promoting a metabolic adaptation towards aerobic fermentative of glucose, negatively regulating the oxidative metabolism in the presence of this sugar to sustain its fast proliferation.
Supported by:FAPERJ (E-26/203.213/2015, E-26/010.001706/2019), ARC (26/010.100.623/2018), CNPq (421676/2017), CAPES (Finance Code 001) **Keywords:**Trypanosoma cruzi;Heme;Energy metabolism.

PV-05 - **Development of a computer visualization tool for the morphological classification of Free-Living Amoebas (FLAs)**

ISSA, M.[1]; BELLINI, N.K.[2]; OLIVEIRA, D.C.[3]; THIEMANN, O.H.[1].
1. INSTITUTO DE FÍSICA DE SÃO CARLOS, USP (IFSC/USP), BRA; 2. INSTITUTO BUTANTAN, BRA; 3. INSTITUTO FEDERAL GOIANO, RIO VERDE - BRA
E-mail: matheus.issa@usp.br

Free-Living Amoebas (FLAs) are unicellular eukaryotic microorganisms widely found throughout nature that belong to the Kingdom Protista. Although the majority of them lives freely in the environment, some genera are facultative opportunistic parasites, such as ***Naegleria*** spp. and ***Acanthamoeba*** spp., which normally cause infections associated with the Central Nervous System (CNS). Due to its high pathogenicity, these infirmities progress so fast that death ensues rapidly, such is the case of Primary Amebic Meningoencephalitis (PAM), caused by ***N. fowleri***, as well as Granulomatous Amebic Encephalitis (GAE), caused by some ***Acanthamoeba*** spp.. Studies related to these microorganisms in Brazil are very scarce, without a precise mapping of its environmental distributions and an optimized method for morphological analysis, increasing the difficulty of efficiently processing samples. Therefore, we are currently elaborating a computer visualization program based on taxonomic keys from the classification guide Page (1988) to be available to the community for future taxonomic identification of FLA. We are collecting the morphological characteristics described in the guide and organizing them into a tabular dataset. This dataset is the input for the software development called Page's Visualization Tool (PVT), which is based on high-dimensional data visualization. Thus, we are proposing a computational tool able to contribute to the optimization of the analysis and improve future research on the morphological characterization of FLAs. **Supported by:**Agência financiadora: FAPESP; Nº do processo: 2021/04364-3 **Keywords:**Free-Living Amoeba;PVT;Page.

PV-06 - **Updated phylogenomic reconstruction of amoebozoan testate amoebae**

LAHR, D.J.G.[1]; DE SOUSA, A.L.P.[1]; TICE, A.[2]; BLANDENIER, Q.[2]; RIBEIRO, G.M.[1]; DUMACK, K.[3]; SINGER, D.[4]; LARA, E.[5]; SOUZA, M.B.G.E.[1]; KLEITZ-SINGLETON, F.[2]; FRY, N.[2]; HENDERSON, T.[2]; BROWN, M.W.[2].
1. USP, BRA; 2. MSU, JACKSONVILLE - USA; 3. KOLN UNIVERSITY - GER; 4. ANGIERS UNIVERSITY-FRA; 5. REAL JARDIN BOTÁNICO DE MADRID, ESP
E-mail: dlahr@ib.usp.br

Shelled (testate) amoebae, unicellular eukaryotes capable of building a shell, appeared multiple times throughout the evolutionary history of life. The eukaryotic 'supergroup' Amoebozoa alone is home to two diverse testate amoebae groups: Arcellinida and Corycidia. Arcellinida is a diverse order of testate amoebae represented by lineages able to build hard shells and have left exceptionally preserved microfossils in the Neoproterozoic, making it a pivotal group to illuminate the early evolution of Eukaryotes. Corycida is a recently established subclade of Amoebozoa represented by the lineages of testate amoebae that produce flexible shells and are distantly related to Arcellinida. Recent efforts of sampling diverse arcellinids and corycids in a phylogenomic framework have resolved the deep phylogenetic relationships within these groups and demonstrate that most major lineages of Arcellinida have already diversified as early as 730 million years ago. Also, a genus of flexible shell testate amoebae (Microchlamys) has found a home within Arcellinida. Despite the recent advance, most genera of Arcellinida and Corycidia remain unsampled, impairing further insights on testate amoebae evolution and classification. Here, we report the new phylogenomic reconstruction of the amoebozoan testate amoebae. We constructed a new phylogenomic dataset (224 genes) of arcellinids and corycids using PhyloFisher, a pipeline that enables the construction of datasets easy to share and update. We included taxa with available phylogenomic data and ten newly sampled taxa, including Microcorycia and Spumochlamys, which produce flexible shells and lack precise placement. Our reconstruction demonstrates that Microcorycia and Spumochlamys are members of Arcellinida, forming a monophyletic clade with Microchlamys. These results show that the diversity within Arcellinida is higher than previously thought, also implying a high diversity of eukaryotic life already in the Neoproterozoic.**Supported by:** 2019/22815-2 FAPESP **Keywords:** amoebae; phylogenetics; evolution.

PV-07 - **Is there a linker histone in *Toxoplasma gondii*?**

SEVERO, V.R.[1]; SOUZA, R.O.O.[2]; VITORINO, F.N.D.L.[3]; DA CUNHA, J.P.C.[3]; ARRIZABALAGA, G.A.[2]; AVILA, A.R.[1]; NARDELLI, S.C.[1].
1. INSTITUTO CARLOS CHAGAS - FIOCRUZ, PR - BRA; 2. INDIANA UNIVERSITY SCHOOL OF MEDICINE, USA; 3. INSTITUTO BUTANTAN, SP BRA.
E-mail: vanessarsevero@gmail.com

The chromatin is a natural barrier to all DNA-dependent processes such as transcription. The chromatin compaction levels are regulated mainly by histones and their post-translational modifications (PTMs) that may act by facilitating or preventing access to DNA. *Toxoplasma gondii* has the four canonical histones (H2A, H2B, H3 and H4), but the fifth histone (H1 or linker histone) has not been identified. In other eukaryotes, H1 links nucleosomes, and its absence could interfere with chromatin condensation. We have identified a small and basic protein in *Toxoplasma*, similar to H1-like of bacteria, which we have named TgH1-like. By immunofluoresce, we were able to locate TgH1-like exclusively in the nucleus of tachyzoite. In addition, using Ultrastructural expansion microscopy (U-ExM) it was possible to observe that TgH1-like accumulates in the nucleolus and nuclear periphery. Performing standard histone extraction protocols, we observed TgH1-like in the same fraction as the histone H4, confirmed by immunoprecipitation assays that also detected histones H3, H2A1, H2Bb and H2B.Z. Knockout parasites showed 22% of vacuoles in asynchronous replication and endopolygeny division. TgH1-like has two post-translational modifications (PTM) sites already described. Parasites mutated at the phosphorylation site (S43A) showed asynchronous division and early karyokinesis events. Mutants for both phosphorylation and ubiquitylation sites (S43A_K45R) showed the same phenotype pattern related to cell division, analyzed using U-ExM. Next, we investigate the nuclei architecture by transmission electron microscopy, where Δ*tgh1-like* showed a decrease in peripheral chromatin compaction. Our results demonstrate that TgH1-like has histone characteristics, playing a role in chromatin compaction and coordination of cell division. To our knowledge, this would be the first linker histone identified in Apicomplexan parasites and will provide new insights into the chromatin dynamics in Toxoplasma. **Supported by:**CAPES, CNPq, Fundação Araucária, Inova-FIOCRUZ **Keywords:**Toxoplasma gondii;Chromatin;Linker Histone.

PV-08 - **The genetic knockout of pyrroline-5-carboxilate synthetase and pyrroline-5-carboxylate reductase reveals a shortcut in the glutamate-proline pathway in Leishmania braziliensis**

CAMPAGNARO, G.D.; CRUZ, A.K..
FACULDADE DE MEDICINA RIBEIRÃO PRETO, USP, RIBEIRÃO PRETO - SP - BRA.
E-mail: campagnarogd@gmail.com

Proline (Pro) is a valuable energy source as its catabolism originates substrates for the tricarboxylic acid cycle. In trypanosomatids, Pro is obtained by uptake from the environment or by *de novo* biosynthesis from glutamate (Glu) via the activity of pyrroline-5-carboxilate synthetase (P5CS) and pyrroline-5-carboxylate reductase (P5CR). *Trypanosoma brucei* is auxotrophic for Pro, whereas *T. cruzi* is able of both Pro uptake and biosynthesis, which unables it to use Glu as sole Carbon source. Starved (4h in PBS) procyclic promastigotes (PRO) of *Leishmania braziliensis*, *L. amazonensis* and *L. major* recovered mitochondrial function with various Carbon sources, including Glu, which suggested a functional *de novo* Pro biosynthesis pathway. Thus, we investigated the functionality of P5CS and P5CR in Pro biosynthesis in *L. braziliensis*. The knockout (KO) of *Lbr*P5CS and *Lbr*P5CR, however, did not affect their ability to efficiently use Glu as energy source. Parasites KO for Pro dehydrogenase, the first catabolic enzyme of Pro, were able to utilize Glu, but not Pro, as sole energy source, indicating that energy generation from Glu follows a separate route from the Pro pathway. Also, parasites starved in PBS and recovered in Glu did not restore their intracellular free Pro levels, indicating that Pro biosynthesis is actually not functional in *L. braziliensis*. Yet, mRNAs encoding for *Lbr*P5CS and *Lbr*P5CR are found in PRO, metacyclic (META) and axenic amastigotes (AMA) forms, but displays highest levels in META. Moreover, myc-tagged *Lbr*P5CS and *Lbr*P5CR showed a variable pattern of expression across different biological forms: whereas *Lbr*P5CS expression is reduced in META and absent in AMA, *Lbr*P5CR expression was detected in all three life cycle stages with highest levels in META. Our results indicate that Pro biosynthesis is not functional in *L. braziliensis* and may suggest that *Lbr*P5CS and *Lbr*P5CR have moonlight functions.
Supported by:FAPESP 2020/02372-6 **Keywords:**Leishmania braziliensis;Glutamate;Proline.

PV-09 - **IDENTIFICATION OF GENES INVOLVED IN SYNTHESIS OF BIOACTIVE PHOSPHOLIPIDS AND MOLECULAR ANALYSIS OF A PLATELET ACTIVATING FACTOR ACETYLHYDROLASE (PAF-AH) OF TRYPANOSOMA CRUZI**

COELHO, F.S.; MOREIRA, I.C.D.F.; TORRES, P.M.; LAZOSKI, C.V.D.S.; VIEIRA, D.P.; LOPES, A.H.D.C.S..
UFRJ, RJ- BRA.
E-mail: lipesc1@micro.ufrj.br

Lysophosphatidylcholine (LPC) is a phospholipid that presents ubiquitous distribution among eukaryotes, including protozoan parasites, such as *Trypanosoma cruzi*. LPC is obtained from phosphatidylcholine (PC) by the action of a cholesterol acetyltransferase or one of the enzymes of the phospholipase A2 (PLA2) family, which includes the platelet-activating factor acetylhydrolase (PAF-AH). LPC acts in various biological and pathophysiological processes, such as inflammatory diseases, especially in atherosclerosis, as well as in innate immunity. Also, *T. cruzi* synthesizes several LPC species, one of which (C-18:1 LPC) behaves similarly to PAF, aggregating platelets and triggering cell differentiation and infectivity of *T. cruzi* towards mouse macrophages. The present study aimed to identify the genes involved in the synthesis of LPC in *T. cruzi* and characterize proteins that participate in the biosynthesis. Enzymes related to the synthesis of LPC were selected from the KEGG pathways database, and a search for their gene sequences was performed on genomic data bank TritrypDB. To confirm the existence of these genes, polymerase chain reactions (PCR) were performed, and the amplified regions were sequenced. Ten genes that code for enzymes of the LPC biosynthetic pathways in *T. cruzi* were amplified using PCR, including a phospholipase A_2 (PLA_2), related to cell invasion events in several parasitical protozoa. Then, we constructed a three-dimensional model of that PLA2, which in fact is a PAF-AH. The PAF-AH activity was detected in the cell extract of *T. cruzi* and a specific PAF-AH inhibitor abolished the activity. In summary, this is the first study to shed light to the molecular structure and function of a PAF-AH in *T. cruzi*. Additional studies will be carried out to determine the role of PAF-AH from *T. cruzi*, as well as its inhibitors, in the invasion of the parasite in mammalian cells. **Supported by:**CNPq, CAPES, FAPERJ and INCT-EM **Keywords:**phospholipids;LPC biosynthesis;phospholipase.

PV-10- **Does *Leishmania* ATM play a role in DNA repair and genome plasticity?**

BLACK, J.A.; DA SILVA, G.L.A.; TOSI, L.R.O..
USP, RIBEIRAO PRETO - SP - BRA.
E-mail: jennifer.stortz@glasgow.ac.uk

DNA damage arises from a myriad of sources leading to a broad spectrum of lesion types including Double Strand Breaks (DSBs). DSBs are considered the most deleterious, and if persistent, can lead to chromosome breakage, genome instability, tumorigenesis, and cell death. In response, eukaryotic cells have evolved a network of pathways known as the DNA Damage Response (DDR). At the forefront of the cellular response to DSBs, is the kinase ATM (Ataxia-telangiectasia Mutated). ATM is recruited to DSBs via the actions of the MRN complex (Mre11-Rad50-Nbs1). Together, these events lead to a phosphorylation cascade promoting the recruitment of repair factors and the establishment of a repair permissive environment. Paradoxically, DSBs can also drive genome diversity, particularly if the DSB is 'controlled', for instance during V(D)J recombination, which underpins immune system diversity. In *Leishmania*, the DDR is less understood. *Leishmania* ATM has been shown to act in response to oxidative stress, but the wider functionalities of this kinase are unknown. Nor is it clear if ATM has functions pertaining to the remarkable plasticity of the *Leishmania* genome; studies implicate DNA damage and DDR activities as drivers of this process. Here, using CRISPR/Cas9 we deleted ATM in *L. major* promastigotes. We found loss of ATM moderately affects growth *in vitro*, but significantly sensitises cells to DSB-inducing genotoxins. Additionally, by sequence analysis, we found evidence of wider genomic alterations (ploidy) and increased instability in ATM's absence suggesting this kinase plays a key role in maintaining *Leishmania* genome stability.
Supported by:FAPESP 2020/01883-7 **Keywords:**Leishmania;ATM;DNA repair.

PV-11 - **Dynamics of TcRab5 isoforms in *Trypanosoma cruzi* epimastigotes**

AMARAL, A.A.; ALCANTARA, C.D.L.; DA CUNHA-E-SILVA, N.L..
UFRJ, RJ- BRA.
E-mail: alexia.achilles@gmail.com

Rab5 is a small GTPase that regulates the budding of vesicles and directs the endocytosed cargo from the plasma membrane to the early endosomes. Its function and dynamics are well established in mammalian cells, but in T. cruzi, which has a peculiar and poorly elucidated endocytic pathway, there is no information but the annotation of two isoforms, TcRab5a and TcRab5b, at the TriTrypDB database. Thus, our goal is to characterize their dynamics and functions. For this, epimastigotes of the Dm28c strain were genetically modified using the CRISPR-cas9 technique. Two populations of mutants were generated by the insertion of the mNeonGreen (mNG) fluorescent protein and the myc peptide genes into the N-terminal region of TcRab5a and TcRab5b. After selection of the transfected parasites, observation of live cells expressing mNG:TcRab5a showed its localization concentrated close to the bottom of the flagellar pocket. In mNG:TcRab5b mutants, signal was found in compartments at the perinuclear region. Endocytosis assays using fluorophore labelled transferrin (Tf), albumin (BSA), ferritin (Ferr) and hemoglobin (Hb), showed that TcRab5a and TcRab5b participate in endocytosis of Tf and BSA but not Ferr and Hb. More than one attempt to obtain knockout mutants for TcRab5a failed, suggesting that these proteins are essential for epimastigotes. On the other hand, we managed to generate TcRab5b KO mutants, and they were unable to endocytose transferrin. Transmission electron microscopy of these cells showed increased number of lipid droplets and lipid inclusions in the lysosome-like organelles and mitochondrial alterations. To evaluate the non-redundant functions of the TcRab5 isoforms, endogenous labeling of the two isoforms in the same cell are under analyses. **Keywords:**Trypanosoma cruzi;TcRab5;Early endosomes.

TB-01 - **EXPANDING THE ARSENAL AGAINST LEISHMANIASIS: NOVEL TAMOXIFEN/CLEMASTINE CHIMERA AS POTENTIAL ANTILEISHMANIALS**

AGOSTINO, V.D.S.[1]; BUERDSELL, M.L.[1]; ULIANA, S.R.B.[2]; DENNY, P.W.[1]; COELHO, A.C.[3]; STEEL, P.[1].
1. DURHAM UNIVERSITY, GBR; 2. ICB-USP, SP - BRA; 3. UNICAMP, SP - BRA.
E-mail: victor.agostino@gmail.com

Collectively, the shortcomings of current treatments make the discovery of new alternative treatments an urgent matter. In this context, tamoxifen, a selective oestrogen receptor modulator (SERM) and known anti-breast cancer drug, has been identified as a potent anti-leishmanial, displaying significant activity against both *in vitro* and *in vivo* infection models. Similarly, clemastine fumarate, an over-the-counter first-generation antihistamine drug, has submicromolar activity against intramacrophage amastigotes of *Leishmania amazonensis* as well as equivalent activity to glucantime in a mouse model infection. Interestingly, both molecules display similar chemical features and have also been proposed to target the same enzyme: the inositol phosphorylceramide synthase (IPCS), an essential enzyme to the parasite which is part of the sphingolipids biosynthetic pathway. However, previous studies have shown that clemastine and tamoxifen have multiple intracellular targets. These could lead to toxicity and lack of selectivity, and are yet to be explored. To investigate this in greater detail – and develop more effective selective compounds –, we have built a library of tamoxifen/clemastine hybrids based on the common chemical features shared by these molecules. Following initial screening against *L. major* and *L. amazonensis* promastigotes, as well as cytotoxicity assays using HepG2 cells, several hybrids have shown submicromolar activity and no toxicity against human cells. This showed an improvement from parental molecules, which are toxic against HepG2 cells. The most active compounds (EC_{50} < 2 μM against both species of promastigotes together with SI > 10 *versus* HepG2) are currently being tested against intracellular amastigotes. This presentation will describe these studies together with the ongoing experiments designed to explore the mode of action and molecular target(s) of these chimeric compounds. **Supported by:**GCRF-CDT (UK)
Keywords: Drug discovery;Treatment;Medicinal chemistry.

TB-02 - **Effect of apigenin *in vitro* and *in vivo* in *Leishmania infantum.***

EMILIANO, Y.S.D.S.; AMARAL, E.E.A.. FIOCRUZ, RJ- BRA.
E-mail: yagoemiliano92@gmail.com

Leishmaniasis, a neglected tropical disease, has been reported in 98 countries and affects 12 million people around the world. *Leishmania infantum* is responsible for the most severe clinical manifestation, visceral leishmaniasis (VL). Current treatment for leishmaniasis is based on pentavalent antimony, amphotericin B and miltefosine, but these treatments have several collateral effects, resistance and therapeutic failure. Apigenin is a flavonoid present in common fruits and vegetables, and it is believed to have several biological functions. In the present study, apigenin demonstrated concentration-dependent inhibition for 72 hours on the *L. infantum* promastigote, reaching an inhibition of 94.6% at its highest concentration used (96 μM), demonstrating an IC_{50} value of 29.9 μM. Its effect was also evaluated in *L. infantum*-infected murine peritoneal macrophages, which were incubated for 72 hours with different concentrations of apigenin (0-24 μM). A concentration-dependent inhibition was observed, reaching 85% and 88% inhibition at 12 μM and 24 μM, respectively. The IC_{50} value of intracellular amastigotes was 2.35 μM. Concerning the murine model of visceral leishmaniasis, *in vivo*, two types of treatments were used, long-term and short-term. BALB/c mice were infected via the peritoneum with *L. infantum* promastigotes ($1x10^9$ cells/ml) and treated with vehicle (control group), apigenin (2 mg/kg/day) or glucantime (100 mg and 200 mg/kg/day). Apigenin demonstrated a reduction of 94% and 99.7% in the parasite load in the liver in the long-term treatment model and in the short-term treatment model, respectively. Furthermore, toxicity was not demonstrated in the Balb/c mice. Taken together, these results suggest that apigenin may be a more efficient and less toxic molecule for the treatment of leishmaniasis.
Supported by:CAPES, CNPq e FAPERJ **Keywords:**Apigenin;Treatment;Visceral Leishmaniasis.

TB-03 - **Effect of MSS as an alternative therapy for the treatment of leishmaniasis**

OZÓRIO, G.G.; EMILIANO, Y.S.D.S.; AMARAL, E.E.A..
FIOCRUZ, RJ- BRA.
E-mail: gabriella.ozorio@gmail.com

Leishmaniasis is a severely neglected tropical disease caused by different species of *Leishmania*. 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 repurposing stands out. In this scenario, we highlight MMS, a drug currently used in the clinic for the treatment of another disease whose activity was described in *Trypanosoma cruzi*. This study evaluated the effect of MMS *in vitro* and *in vivo* and its possible mechanism of action. Promastigotes of *L. infantum* and *L. amazonensis* were treated with different concentrations of MMS (18,75 µM – 1200 µM) for 24 and 72 hours and demonstrated an inhibition of cellular viability in a concentration-dependent manner. To investigate a possible mechanism of action, promastigotes treated with MMS showed an increase in ROS levels in both species at 24 and 72 hours, and the preincubation with antioxidant molecules were not capable of protecting cells from the inhibition promoted by MMS. Against the intracellular amastigote, MMS (4 – 280 µM) demonstrated an inhibition of the infection index in a concentration-dependent manner after 72 hours of treatment and proved to be not toxic in the macrophage toxicity assay. In the *in vivo* study, BALB/c mice were infected with *L. infantum* promastigotes for 7 days and treated with 1, 5, 3 or 6 mg/kg/day of MMS, 100 mg/kg/day of glucantime or the vehicle. MMS was able to decrease the parasite load in the liver of infected mice compared to the control and Glucantime 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 MMS is a good possibility for leishmaniasis chemotherapy. **Supported by:**CNPq; CAPES; Faperj; IOC/Fiocruz **Keywords:**Leishmaniasis;drug repurposing;Mechanism of action.

TB-04 - **2'-hydroxyflavanone effects against wild type and antimony-resistant *L. infantum*, toxicity and pharmacokinetics**

DE OLIVEIRA, L.G.F.; OZÓRIO, G.G.; PAES, T.F.; DOS SANTOS, J.P.R.; DA SILVA, J.D.P.A.; MACHADO, M.P.; SILVA, A.C.A.; DA SILVEIRA, G.P.E.; PEREIRA, H.M.; PINTO, D.P.; DA CUNHA JUNIOR, E.F.; AMARAL, E.E.A..
FUNDAÇÃO OSWALDO CRUZ, RJ- BRA.
E-mail: luizagervazoni@ig.com.br

Leishmaniasis deserves attention due to the wide variety of associated clinical manifestations and their high annual incidence. Although there are many drugs available as alternatives for leishmaniasis treatment, they remain mostly ineffective, toxic, expensive and longstanding, in addition to the resistance cases reported over the years. Among the search for new alternatives to treat this disease is the search for new medicines from natural sources. Studies have demonstrated the leishmanicidal effect of flavonoids, a class of plants secondary metabolites that have antioxidant and anti-inflammatory activity described in several diseases. 2'-Hydroxyflavanone (2HF) is a flavanone currently known for its activity in tumor cells. Previous results demonstrated 2HF *in vitro* and *in vivo* activity against wild-type and antimony-resistant *L. amazonensis*. Due to the promising effects of 2HF and the drug development process, this study evaluated 2HF against wild-type and antimony-resistant *L. infantum* and its toxicological and pharmacokinetic parameters. 2HF was able to inhibit *L. infantum* wild-type promastigotes and amastigotes in a concentration-dependent manner after 72 h as well as antimony-resistant promastigotes. In acute and subacute preclinical toxicity, 2HF proved to be safe, showing no mortality or changes in weight gain and water/food consumption. An LC–MS/MS selective and sensitive analytical method was developed for 2HF detection and quantification. This method was validated according to ANVISA guidelines. To determine the pharmacokinetic profile, BALB/c mice received a single oral dose of 2HF, and blood was collected at different times. 2HF pharmacokinetic parameters were calculated using a noncompartmental mathematical model. 2HF demonstrated a Tmax of 5 min and a T ½ of 97.52 min with a Cmax of 2HF of 185.86 ng/mL. Taken together these results indicate 2HF promising effects against *L. infantum* and point out further in vivo studies with different formulations. **Supported by:**FAPERJ; CNPq; CAPES; PAPES; IOC/FIOCRUZ **Keywords:**Leishmania;Flavonoid;Pharmacokinetics.

TB-05 - **Innovative microfluidic device to synthesize amphotericin B-loaded polymeric nanoparticles for cutaneous leishmaniasis treatment**

MOTA, G.C.N.[1]; SUZUKI, É.Y.[1]; BERGMANN, B.R.[1]; BALBINO, T.A.[2]; SOUSA-BATISTA, A.D.J.[2].
1. INSTITUTO DE BIOFÍSICA CARLOS CHAGAS FILHO – UFRJ, BRA; 2. PROGRAMA DE PÓS-GRADUAÇÃO EM ENGENHARIA DA NANOTECNOLOGIA – PENT / COPPE – UFRJ, RJ- BRA
E-mail: giovanna@biof.ufrj.br

Cutaneous leishmaniasis (CL) treatment is based on multiple injections with drugs that produce inadmissible systemic toxicity. Therefore, effective local treatment is highly desirable. Previously, we demonstrated that polymeric nanoparticles loading amphotericin B (NP-AmB) as produced by conventional nanoprecipitation have shown promising efficacy by intralesional treatment. However, that is not a reproducible and industrially scalable method. Thus, we proposed to develop NP-AmB by microfluidic-assisted nanoprecipitation that employs precise mixing conditions, and is of easy scale-up. To reduce costs, we manufactured clean-room free and reproducible 3D devices, unlike the commercially available devices. First, NPs were produced by varying their TFR (Total Flow Rate) and FRR (Flow Rate Ratio). The optimized condition for lower size distribution (< 0.3) was FRR= 0.225 and TFR= 2000 µL/min. That yielded blank NPs with 247 nm and PDI = 0.076, and NPs-AmB with 148 nm and PDI= 0.253. Mouse macrophages were treated with NP, NP-AmB or free AmB for cytotoxicity studies. Blank NP CC50 was > 100 ug/mL, whereas NP-AmB and free AmB CC50 were 30 ug/mL and 2 ug/mL, respectively, demonstrating a 15-fold reduced toxicity of AmB after nanoencapsulation. These results indicate the potential use of NP-AmB for a safer local treatment against CL with AmB employing an industrially scalable manufacturing process. **Supported by:**Vale
Keywords: AmphotericinB;Leishmaniasis;Nanoparticles.

TB-06 - **CHALCONE NANOCRYSTALS FOR ORAL TREATMENT OF CUTANEOUS LEISHMANIASIS**

BORSODI, M.P.G.[1]; LIMA, W.P.[2]; MENEZES, J.C.V.[1]; DE OLIVEIRA, D.E.[1]; COSTA, N.D.A.[1]; CABRAL, L.M.[1]; DA SILVA, A.J.M.[1]; STEEL, P.[3]; SOUSA-BATISTA, A.D.J.[1]; BERGMANN, B.R.[1].
1. UFRJ, RJ- BRA; 2. UNIGRANRIO, RJ- BRA; 3. DURHAM UNIVERSITY, DURHAM – ENG
E-mail: mariapaula@biof.ufrj.br

Treatment of cutaneous leishmaniasis (CL) needs safer and more active drugs especially for non-invasive use. We have synthesized a synthetic trimethoxylated chalcone (NAT22) showing high activity (IC_{50} = 0.5 µM, SI =13) against *Leishmania amazonensis.* However, NAT22 is poorly soluble in water due to its large crystals (~200 µm), hampering its oral use. Here, we aimed to reduce the size of NAT22 crystals by nanomilling in order to improve activity and oral efficacy. For that, NAT22 crystals were submitted to dry milling followed by wet milling yielding nanocrystals (nanoNAT22). Promastigotes were incubated with different concentrations of NAT22 or nanoNAT22 for 72 h, when cell viability evaluated by resazurin. For anti-amastigote activity, bone marrow-derived macrophages were infected with promastigotes (1:10) for 24 h at 37 °C and then treated for further 48 h with nanoNAT22 or NAT22 and cytotoxicity was assessed by LDH release in supernatants. NanoNAT22 in aqueous medium proved to be 18-times more active than NAT22 in the same medium. As expected, they were equally active when pre-solubilized in DMSO for both promastigotes (IC50 = 0.7 µM) and intracellular amastigotes (IC50 = 0.5 µM). The selectivity index was higher for nanoNAT22 (SI = 20) than NAT22 (SI = 13). For *in vivo* studies, BALB/c mice were infected in the ear with *L. amazonensis*-GFP. Seven days later, were orally treated daily (40 mg/kg) with nanoNAT 22 or NAT22 for 5 weeks in propylene glycol. Controls received intralesional Glucantime in PBS (1.5 mg/kg, 1x/week). Lesion sizes were measured 2x/week. On day 52 of infection, ear parasites were measured by fluorimetric and limiting dilution assays. Oral nanoNAT22 was more effective than NAT22, reducing parasitic load by 52% and 30%, respectively, comparable to injectable Glucantime. In conclusion, nanomilling significantly improves solubility, anti-parasitic selectivity, oral bioavailability and efficacy of NAT22 chalcone against CL. **Supported by:** VALE DO RIO DOCE **Keywords:** CHALCONE; NANOCRYSTALS; LEISHMANIA.

TB-07 - **MMV Pandemic box in vitro screening identifies new compounds highly active against the tachyzoite stage of *Toxoplasma gondii***

DOS SANTOS, M.; VAZ, G.H.D.S.; DE SOUZA, G.C.A.; VITOR, R.W.D.A.; MARTINS-DUARTE, É.D.S..
UNIVERSIDADE FEDERAL DE MINAS GERAIS (UFMG), BH- MG - BRA.
E-mail: mikesnts@outlook.com

Toxoplasmosis is an asymptomatic infection for most of the population, however, immunocompromised patients may evolve with ocular disorders and/or Central Nervous System involvement. Despite its importance, current chemotherapies are exclusively for the acute phase of the disease. In addition, the treatment presents therapeutic failures, teratogenicity, and frequent side effects, leading to low adherence. Thus, the development of new therapies that circumvent these problems has been a challenge. The Medicines for Malaria Venture (MMV), aiming to foster the development of new therapies for diseases with pandemic potential, has created the Pandemic Response Box. The box contains 400 compounds, which are either already in clinical use or development. To evaluate their activity against T. gondii, the 400 compounds were screened by treating tachyzoite infected cells for 7 days with 1 μM. Of the 400 compounds, 24 were able to inhibit proliferation by more than 80%. IC50 determination showed that 8 drugs/compounds inhibit T. gondii tachyzoite proliferation in concentrations lower than 100 nM and with selective index against the host cell. Further analysis showed that the different compounds have different modes of action, including both an immediate effect at the first replication cycle (48h of treatment) and a delayed effect (> 48h of treatment). The compounds that showed an immediate effect against the parasite replication had its mode of action studied by transmission electron microscopy (TEM) after 48h of treatment. TEM analysis confirmed that treatment with these compounds drastically affected tachyzoite morphology, inhibiting both its proliferation and leading to cell death. Thus in this study, we show new active drugs with potential for the future treatment of the acute stage of toxoplasmosis. **Supported by:**FAPEMIG, CNPq e PRPq-UFMG **Keywords:**Toxoplasmosis;Drug repoditioning;New therapies.

HP-04 - **Cytokine analysis associated with bioluminescent imaging to the follow up of mice infected with Leishmania amazonensis.**

HENRIQUES, C.[1]; CASCABULHO, C.[1]; GODINHO, J.L.P.[2]; DE SOUZA, W.[2]. 1. INSTITUTO OSWALDO CRUZ, RJ - BRA; 2. UFRJ- RJ - BRA.
E-mail: cristina.henriques@fiocruz.br

The outcome of leishmaniasis caused by *Leishmania amazonensis,* depends on the dynamic modulation of chemokines and cytokines expression, by cells involved in the cellular response. Thus, the protozoan establish the infection and the lesion progresses. Here we report data on cytokine identification in the serum and lesion of mice infected with *L. amazonensis* wild type (LamaWT) and genetically modified parasites (GM),expressing fluorescent proteins (LamaEGFP and LamaCherry) and firefly luciferase (LamaLUC). The infection was performed, at the base of the tail (10^8 promastigotes), which displays a protuberant lesion that stands out under the skin, facilitating the evaluation of lesion progression. In the lesion, TNF-α, IFN-ɣ and MCP-1 could be detected by flow cytometry at the 20 and 50 dpi. Mice infected with LamaWT displayed at least two fold higher MCP-1 levels than mice infected with LamaLUC, LamaEGFP and LamaCherry. MCP-1 levels dropped off only in the LamaWT at the 50dpi, and IFN-ɣ dropped off in the LamaWT and LamaLuc strains. In opposition, IL-6 levels in the blood of mice infected with LamaWT and LamaLUC, increased from 20 dpi to 50 dpi. TNF-α levels in mice infected with LamaWT, LamaEGFP and LamaCherry strains at the 20 dpi were significantly lower than the levels in the lesion of mice infected with LamaLUC strain. TNF-α increased significantly from the 20 dpi to the 50 dpi, in the LamaWT strain. The follow up of infection caused by LamaLUC in mice, was evaluated by bioluminescent imaging. The photons emitted by the bioluminescent LamaLUC, inside the lesion, at the top of the tail, are promptly available, which could give us in real time information about the *Leishmania* growth and lesion progression. The association of bioluminescent imaging with cytokine evaluation, in the blood and in the lesion by flow cytometry, is an improvement in the follow up of disease/ lesion progression and for pre-clinical studies. **Keywords:**Leishmania amazonensis;luciferase;cytokines.

HP-07 - **Acquired Toxoplasma gondii infection leads to retinal microvascular abnormalities in mice.**

DE CAMPOS, V.S.; DA ROSA, B.G.; SOMMEVILLE, T.; DA COSTA, K.C.; ADESSE, D.P..
FIOCRUZ, RJ - BRA.
E-mail: vvncampos@gmail.com

Toxoplasmosis affects one third of the world population and its etiologic agent is the protozoan *Toxoplasma gondii*. *T. gondii* has a tropism for 3 specific organs (muscle, brain and eyes) and ocular toxoplasmosis (OT) is well described in the literature. Retinochoroiditis is identified as the main manifestation of OT, but vascular occlusion can occur, even away from active lesions, which may result in hemorrhages. In addition, cases of retinal detachment and subretinal neovascularization have been reported. Our group demonstrated microcirculatory alterations and reduced angiogenesis in mouse brains after acquired *T. gondii* infection, suggesting that such alterations may also occur in OT. This work aims to analyze the effects of acquired *T. gondii in vivo* infection on the retina and its vasculature. For acquired ocular toxoplasmosis model, C57Bl6 mice were inoculated intragastrically with 2 ME49 strain cysts and analyzed at 10, 20 and 30 days post infection (dpi). We observed that infection led to body weight loss and reduced food consumption at all times studied. Cytometric bead array analysis showed a transient increase in serum TNF-α, IFN-g and IL-6 levels at 10 dpi. Confocal microscopy analysis of retinal flat mounts revealed a reduction in the interaction between endothelial cells and astrocytes in infected mice at 10 dpi. At 20 and 30 dpi, we observed an influx of immune cells in the superficial plexus of the retina, which is not seen at 10 dpi, in addition to an apparent astrogliosis. Expression of pro-inflammatory- and angiogenesis-related genes by RT-qPCR revealed an increase in CX3CR1, marker of microglial cells and a reduction in DLL4-NOTCH1 genes, part of angiogenic signaling cascade. Our data suggest that *T. gondii* infection induces an increase in retinal inflammatory profile that would lead to damage to the retinal vasculature, thus contributing to visual impairment in toxoplasmosis. **Supported by:**INOVA no. 3231984391; CNPq 401772/2015-2 - 444478/2014-0;INCT/INNT 465346/2014-6 for KC ,FAPERJ no. E-26/010-001199/2015; Projetos Temáticos no. E26/010.101037/201; Sediadas grant no. E-26/010.001493/2019 for KC). **Keywords:**Ocular Toxoplasmosis;Vasculature;Retina.

HP-12 - **Skeletal muscle pathology and atrophy in mice infected with *Toxoplasma gondii***

VIEIRA, P.D.C.; HORITA, S.I.; CASCABULHO, C.; BARBOSA, H.S.; ADESSE, D.P..
FIOCRUZ, RJ - BRA.
E-mail: paloma.vieira.rj@gmail.com

Skeletal muscle tissue (SkM) is critical for the transmission of *Toxoplasma gondii* where it survives as tissue cysts and causes myositis in patients. However, little is known about how the infection can affect the SkM and its physiology. The aim of this work is to study SkM physiology and the impact of *T. gondii* infection in muscle fibers. For this purpose, Swiss Webster mice were inoculated intragastrically with *T. gondii* Me49 strain cysts and evaluated up to 40 days post infection (dpi). At 10 dpi, a decrease in the body weight was identified and, although no differences were observed in weightlifting and Kondziella's tests, functional grip test showed decreased time of applied force for infected animals. The oxidative muscle soleus (SO) and glycolytic tibialis anterior (TA) were collected for analysis. Parasites were identified in SkM fibers by histochemistry and RT-PCR. Reduced myofibers and inflammatory infiltrates were found in TA both at 10 and 40 dpi. Fiber distribution of the muscle showed a predominance of smaller fibers at 10, but not at 40 dpi. Interestingly, increased expression of atrophy-related genes *Murf1* and *Atrogin1* were only identified in infected TA. at 40 dpi. Regeneration was observed by the presence of myofibers with centralized nuclei, which were 12% of total fibers in TA. Pax7 expression, however, was reduced at 10 dpi and did not change after 40 dpi in TA. In SO, Pax7 was increased after 40 dpi indicating a late regenerative process. Mitochondrial fusion (Opa1) and fission (Fis1) genes were increased only in SO at 40 dpi. Infection induced a systemic increase of IL-6, IFN-γ and TNF-α at 10 dpi, which was normalized at 40 dpi. Because IL-6 signaling pathway is relevant for muscle atrophy, we evaluated the expression of IL-6 receptor (*IL6ra*), and *stat3*. *IL6ra* and *stat3* had increased expression at 40 dpi only in SO. *T. gondii* differentially affects muscle fibers according to metabolic features leading to functional defects. **Supported by:**INOVA Fiocruz, IOC, CNPq, Edital PAPES VII, Faperj **Keywords:**Toxoplasma gondii;Skeletal muscle;Muscle atrophy.

HP-14 - **Impact of nutritional status on the infection course and experimental treatment of *Leishmania infantum*-infected mice**

PAES, T.F.; CAPILLA, L.G.; DE LUCA, P.D.M.; AMARAL, E.E.A..
FUNDAÇÃO OSWALDO CRUZ, RJ- BRA.
E-mail: taianapaes@hotmail.com

Remoto Poster Session

Malnutrition and visceral leishmaniasis (LV) are major public health problems since both are responsible for millions of deaths in many countries. The development and progression of leishmaniasis is associated with the host immune response type. In this scenario, malnutrition can directly affect the course of leishmaniases, as it impairs several components of the immune system. It has already been reported that malnutrition directly interferes with immunity and/or susceptibility to LV. However, there is little information about the effects of malnutrition on the LV treatment response in infected hosts. In this context, the objective of this work was to evaluate the influence of nutritional status on the treatment of mice subjected to experimental malnutrition and refeeding and infected with *Leishmania infantum*. Weaned BALB/c mice that received the control diet or restricted diet were infected or not and treated or not with glucantime. The nutritional status of the mice was evaluated through phenotypic markers and hematological and biochemical parameters. The infection effects were evaluated through an LDA assay. Our results showed that the restricted diet was able to induce mild malnutrition. Malnourished mice demonstrated significant weight loss, slow body growth and low body mass index (BMI) values, while those that were refeeding began to grow and recovered their body weight and BMI. Malnourished and refed mice presented a low parasitic load in the spleen. In the liver, both groups presented a high parasitic load, and when the malnourished BALB/c mice were treated with glucantime, they showed a smaller decrease in parasitic load than treated control mice. In conclusion, a restricted diet was able to generate mild malnutrition and cause changes similar to those observed in marasmic malnutrition in the nutritional status of mice. During LV, data suggest that malnutrition impaired the treatment, and the refeeding process was not able to fully reverse this effect. **Supported by:**CAPES, CNPq and FAPERJ **Keywords:**Visceral leishmaniasis;Marasmatic malnutrition ;treatment.

HP-19 - **Interleukin-10 enhances parasite burden in *Leishmania (L.) major* infected macrophages *in vitro*, but not in *Leishmania (V.) braziliensis*.**

SOARES, S.A.E.; DE SOUZA, V.A.; DE OLIVEIRA, M.A.P..
UNIVERSIDADE FEDERAL DE GOIÁS, BRA.
E-mail: sam.espellet@gmail.com

INTRODUCTION: Leishmaniasis is a disease with high incidence in tropical countries and is an important concern for public health agencies. *Leishmania (V.) braziliensis* (Lb) is the main species causing cutaneous leishmaniasis in Brazil and *Leishmania (L.) major* (Lm) is the best-described species causing cutaneous leishmaniasis in murine models. Macrophages are the main cells parasitized by the protozoan and classically activated macrophages (cMo) by TH1 stimuli are related to the control of the parasite load. Alternatively activated macrophages (AAMo), stimulated by IL-4 and/or IL-10 are susceptible to Lm parasite, but there is no information about susceptibility of Lb *in vitro*. OBJECTIVE: Compare Lb and Lm survival *in vitro* in murine macrophages stimulated with IL-4 and/ or IL-10. METHODS: Macrophages from BALB/c mice elicited with thioglycolate were stimulated or not with IL-4 or IL-10 for 24h and infected with Lb or Lm. Presence of AAMo was evaluated by arginase activity assay and parasite load was determinated by counting amastigotes in light microscopy after 3, 24, 72 and 120 hours post-infection and by the recovery of promastigote forms in culture assay. RESULTS: There was a significant decrease in the parasite load after 72h in Lb infected macrophages in all unstimulated, IL-4 and IL-10-stimulated macrophages assayed by microscopy or promastigote recovery. This phenomenon contrasted with infections with Lm, which had an increase in parasite load after 72h in infections in unstimulated macrophages and in macrophages stimulated with IL-4 or IL-10 with an increase in parasite load in infections of cells stimulated with IL-10 in relation to the unstimulated. CONCLUSION: Control Lb occurs even in IL-4 or IL-10 stimulated macrophages. On the other hand, macrophages were susceptible to infection by Lm, alternative activation favored an increase in the parasite load. **Supported by:**FAPEG, CAPES, CNPq, INCT-IPH. **Keywords:**Murine macrophage;cutaneous leishmaniasis;Alternative activation.

HP-20 - **Nutritional and oxidative stress induce AMPK phosphorilation in Trypanosoma cruzi**

SILVA, M.A.; COSTA, M.A.V.; SCHENKMAN, S..
UNIVERSIDADE FEDERAL DE SÃO PAULO, SP- BRA
E-mail: michel.aug.silva@gmail.com

Trypanosoma cruzi demonstrates great evolutionary and adaptive capabilities in its biological cycle according to the host due to variable nutritional and oxidative conditions. Therefore, it needs several metabolic pathways and mechanisms that allow it to develop and maintain its cellular stability. Adenosine monophosphate-activated protein kinase (AMPK) is an enzyme that acts as a central regulator of energy homeostasis in many organisms. AMPK is a heterotrimer formed by two regulatory subunits (β and γ) and a catalytic subunit (α). It is mainly regulated by changes in AMP in cells and in the active state, a conserved loop in the α chain is phosphorylated. Therefore, we investigated in which conditions AMPK is phosphorylated in *T. cruzi*. We found that AMPK was rapidly phosphorylated under nutritional and oxidative stresses, generating two different products recognized by a monoclonal anti-phospho-threonine 172, an epitope conserved in the α1 chain of the parasite kinase. In the first case, the phosphorylation occurred in a protein that migrate faster in SDS-PAGE and follows an increase in the AMP/ATP levels. In the second there was no change the AMP/ATP ratio and the product of phosphorylation migrate slower. We concluded that different stress types caused variable changes in the AMPK phosphorylation and possibly function, which might be relevant for parasite adaptation. **Supported by:**FAPESP 2021/12527-0 **Keywords:**AMPK;Stress response;Kinase.

HP-21 - **Serum antibodies blocked by glycan antigens in canine visceral leishmaniasis serology are mostly IgA immune complexes**

DE CARVALHO, C.A.[1]; HIRAMOTO, R.M.[2]; MEIRELES, L.R.[3]; DE ANDRADE JR, H.F.[3].
1. FACULDADE DE MEDICINA DA USP, SP - BRA; 2. INSTITUTO ADOLFO LUTZ, SP - BRA; 3. INSTITUTO DE MEDICINA TROPICAL DA FACULDADE DE MEDICINA DA USP, SP - BRA.
E-mail: camilacarvalho@usp.br

Immune complexes (ICs) are found in canine visceral leishmaniasis (CVL) and interfere with the serum detection of antibodies. Dissociation of these monovalent complexes by dissociative enzyme-linked immunosorbent assay (ELISA) removes false-negative results and allows some characterization of antibodies and antigens. We studied the serology of dogs with suspected CVL in an endemic area, testing two Leishmania (Leishmania) [L. (L.)] infantum antigens. We analysed the presence of immunoglobulin G (IgG), immunoglobulin A (IgA) and immunoglobulin M (IgM) antibodies specific to promastigote soluble extract (PSE) and low-molecular weight glycans (glycan–bovine serum albumin (BSA) complex – GBC) by conventional and dissociative ELISA. Our results showed a significant fraction of IgA ICs (46.5% for PSE and 47.6% for GBC), followed by IgG ICs (10% for PSE and 23.5% for GBC). IgM ICs were more frequent for PSE (22.7%). Hypergammaglobulinaemia in CVL would be related to the presence of IgA and IgG ICs, resulting in deficient elimination of these antibodies. Our data confirmed the presence of ICs that can generate false-negative results in conventional serology. The production of IgA antibodies and the high frequency of blockade by glycan antigens suggest the active participation of this immunoglobulin and its ICs in the immunopathology of CVL, indicating a new path for further Research **Supported by:**FAPESP - PROCESSO 2017/14675-0 **Keywords:**Immune complexes;Antibodies;Glycan.

HP-22 - ***In vitro*** **evaluation of aldimine derivatives as drugs for the treatment of visceral leishmaniasis**

COSTA, A.F.P.[1]; OSTOLIN, T.L.V.D.P.[1]; CARDOSO, J.M.D.O.[1]; MOREIRA, G.J.L.[1]; GUSMAO, M.R.[1]; GONÇALVES, L.C.[1]; COSTA, J.A.[1]; DE FATIMA, A.[2]; OLIVEIRA, B.G.D.F.[2]; REIS, A.B.[1]; SOARES, R.D.D.O.A.[1]; ROATT, B.M.[1].
1. UFOP, MG - BRA; 2. UFMG, MG - BRA.
E-mail: ana_pcosta@yahoo.com.br

Visceral leishmaniasis (VL) is the most serious form of leishmaniasis, which if not diagnosed and treated in time leads to death. Today, the available restrict treatment arsenal consist of few drugs, such as pentavalent antimonials, paramomycin, pentamidine, miltefosine, amphotericin B deoxycholate and liposomal. But due to some limitations, such as the adverse effects observed in the treatment and that there is still no ideal therapy, the World Health Organization (WHO) recommends and supports the investigation of new drugs and treatment strategies against the disease, such as the study of new compounds in order to broaden perspectives for the development of more effective therapies. In this sense, synthetic compounds such as aldimines, emerge as possible alternative. The main objective was to test the leishmanicidal/leishmaniostatic action of aldimine derivatives 3H8 and 3D7, compounds with known antimicrobial action, in *L. infantum* infected murine cells (RAW 264.7 lineage). For this study, the OP46 strain of *L. infantum* genetically modified with a fluorescent gene (OP46 GFP^+) was used. We evaluated the cytotoxicity of different compounds by the colorimetric method of 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) in murine macrophages, with a lower toxicity observed at 3H8 when compared to the compound 3D7. For the *in vitro* evaluation of leishmanicidal activity in amastigotes, RAW 264.7 murine macrophages infected with OP46 GFP^+ promastigotes were used and treated with different chemical compounds for 24, 48 and 72 h, being evaluated by flow cytometry. Our results showed a decrease in the parasite load in relation to the control infected group, regardless of the time of infection, with both compounds. The dataset obtained suggests that the aldimine compounds evaluated have leishmanicidal potential and deserve to be evaluated in experimental *in vivo* chemotherapy studies against VL. Acknowledgments: UFOP, PROPPI, CAPES, CNPq, FAPEMIG, INCT-DT. **Supported by:**FAPEMIG APQ-02256-18 CAPES finance code 001 **Keywords:**Visceral Leishmaniasis;Treatment;Aldimines.

HP-23 - **Unraveling the mechanisms of cholesterol scavenging by intracellular amastigotes of Trypanosoma cruzi**

ALCANTARA, C.D.L.; PEREIRA, M.G.; DE SOUZA, W.; DA CUNHA-E-SILVA, N.L..
INSTITUTO DE BIOFÍSICA CARLOS CHAGAS FILHO/UFRJ, RJ - BRA.
E-mail: alcantara@biof.ufrj.br

Trypanosoma cruzi amastigotes develop intracellularly in the vertebrate host. These forms live in the cytosol of the host cells where they have full access to host cytosolic macromolecules and organelles. Although amastigotes have a complete and functional endocytic pathway, data about which macromolecules they can endocytose and how they interact with host organelles are scarce. Cholesterol performs most of the sterol found in intracellular amastigotes suggesting that they can scavenge cholesterol from the host cell. However, the mechanisms utilized by amastigotes to capture cholesterol while inside the host cell cytosol are still to be determined. In this work, we investigate cholesterol traffic in infected host cells by using fluorescent cholesterol (TFChol) tracer, confocal and super-resolution microscopy analysis, and high-resolution electron microscopy. For this, infected host cells (24hpi) were incubated with TFChol added directly to the culture medium or preloaded in LDL particles. By confocal microscopy, TfChol were found in punctual structures at the anterior region of the amastigotes, close to the flagellar pocket/cytostome . The appearance of these structures inside the parasite was earlier when the TfChol was added directly to the culture medium, suggesting that the traffic from host membrane constitutes a faster cholesterol route of acquisition by the parasites. Antibody labeling of host cell organelle molecular markers showed participation of the endoplasmic reticulum in this fast route of cholesterol acquisition. For analysis at ultrastructural level we used an anti-Bodipy antibody, to label TFChol, and fluoronanogold particles as secondary antibodies to correlate localization of TfChol labelling in light microscopy with amastigotes intracellular structures using transmission electron microscopy. This analysis will give us more insights into the cholesterol traffic from the host to the amastigotes, unraveling a possible transfer via contact-sites. **Supported by:**Faperj, CNPq **Keywords:**amastigotes;cholesterol traffic;endocytosis.

HP-24 - **Disruption of the Inositol Phosphorylceramide Synthase gene affects *Trypanosoma cruzi* differentiation and infection capacity**

DOS SANTOS, N.S.A.[1]; DE MACEDO, J.P.[1]; CHAME, D.F.[1]; DENNY, P.W.[2]; TEIXEIRA, S.M.R.[1].
1. UFMG, MG - BRA; 2. DURHAM UNIVERSITY, GBR
E-mail: nailmaaprigio@hotmail.com

Sphingolipids (SLs) are essential components of all eukaryotic cellular membranes. In fungi, plants and 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. Since fungal IPC synthase (IPCS) null mutants are not viable and IPCS activity has been described in all parasite forms of *T. cruzi*, this enzyme has been considered an ideal target for the development of new and more effective drugs to treat CD. IPCS is an integral membrane protein conserved among 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 showed that the lack of IPCS activity does not affect epimastigote proliferation or its susceptibility to drugs 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, proliferation of intracellular amastigotes as well as 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 were unable to cause infection *in vivo*, even in a highly susceptible animal model of infection. **Supported by:**CNPq - Processo: 380642/2022-0 **Keywords:** Trypanosoma cruzi ;Sphingolipids; phosphorylceramide synthase.

HP-25 - **Ribozyme mediated knockdown of Jumonji-like C2 histone demethylase (PfJmjC2) decreases steady-state variant gene transcripts in the malaria parasite *Plasmodium falciparum***

WUNDERLICH, G.[1]; SILVA, T.M.[2]; BESENBRUCH, A.P.[1]; ARAUJO, R.B.D.[3]; MARTINS JR, J.[4].
1. INSTITUO CIÊNCIAS BIOMÉDICAS, USP, BRA; 2. NATIONAL INSTITUTES OF HEALTH - USA; 3. INSTITUTO DE CIÊNCIAS BIOMÉDICAS, USP, SP - BRA; 4. INSTITUTO DE QUÍMICA, USP, SP - BRA.
E-mail: gwunder@usp.br

The persistence of the malaria parasite *Plasmodium falciparum* depends strongly on its capacity to evade the human host's immune response and this is mediated by the deposition of large parasite encoded proteins on the infected erythrocytes surface. These antigens were termed *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) and are encoded by the *var* gene family. Due to the fact that these proteins become targets of the acquired immune response, their expression is controlled in a tight manner. This means that only one or two PfEMP1 per infected red blood cell is expressed - from a repertoire of up to 90 different *var* alleles present in the genome. The control of the PfEMP1-encoding *var* genes occurs at the transcriptional level and depends on multiple factors including chromatin writers, readers and erasers. In order to elucidate participating factors, we created a *P. falciparum* parasite line where the histone code modifier PfJmjC2 can be controlled by the action of a 3' inserted ribozyme. Upon knockdown of the PfJmjC2 transcript, less transcript was detected by qPCR, as expected. The knockdown led to no discernible growth phenotype, although only a fraction of HA-tagged PfJmjC2 could be detected compared with controls. When monitoring *var* transcripts in parasites phenotype-selected for the expression of determined *var* genes, we observed the downregulation of some - but not all - of the actively transcribed *var* genes. Global RNAseq analysis revealed that upon PfJmjC2 depletion, the transcripts of many regulatory proteins including PfAP2 proteins and other known histone modifiers (SET, SIR2A) were decreased. The same was true for invasion-related protein-encoding genes. We conclude that JmjC2 is an important regulator in a network of pathways, however, its function is probably redundant or timely compensated for due to the fact that knockout is possible and no growth phenotype under knockdown was observed. **Supported by:**FAPESP 2017/24267-7 **Keywords:**Plasmodium;chromatin modifier;transcript knockdown.

HP-26 - **Evaluation of immunogenicity and efficacy potential of a polyepitope T-cell vaccine candidate against visceral leishmaniasis in a murine model**

OSTOLIN, T.L.V.D.P.[1]; GUSMAO, M.R.[1]; MATHIAS, F.A.S.[1]; CARDOSO, J.M.D.O.[1]; SANTOS, A.D.C.F.[1]; ROATT, B.M.[1]; SOARES, R.D.D.O.A.[1]; RESENDE, D.D.M.[2]; RUIZ, J.C.[2]; DE BRITO, R.C.F.[1]; REIS, A.B.[1].
1. UFOP, MG - BRA; 2. INSTITUTO RENÉ RACHOU (FIOCRUZ), MG - BRA.
E-mail: thais.ostolin@gmail.com

The development of an immunogenic, effective, and safe vaccine is essential as an alternative for disease control. The present study aimed to evaluate the immunogenicity and efficacy potential of a polyepitope T-cell antigen candidate against visceral leishmaniasis in a murine model. BALB/c mice were immunized with three doses subcutaneously with Poly-T Leish alone or adjuvanted with Saponin plus Monophosphoryl lipid A, with 15-day intervals between doses, and challenged with 10^7 stationary-phase *Leishmania infantum* promastigotes. Immunogenicity and parasitism in spleen and liver of immunized mice were evaluated 45 days post-challenge. Our results revealed that the Poly-T Leish and Poly-T Leish/SM increases the percentage of specific T ($CD4^+$ and $CD8^+$) lymphocytes proliferation. Also, Poly-T Leish and Poly-T Leish/SM induced a high percentage of T cells producing IFN-γ and TNF-α, meanwhile, the Poly-T Leish/SM group also showed an increased percentage of multifunctional T cells producing double ($IFN\text{-}\gamma^+TNF\text{-}\alpha^+$, $TNF\text{-}\alpha^+IL\text{-}2^+$, $IFN\text{-}\gamma^+IL\text{-}2^+$) and triple-positive ($IFN\text{-}\gamma^+TNF\text{-}\alpha^+IL\text{-}2^+$) cytokines. Poly-T Leish and Poly-T Leish/SM stimulated a decreased IL-4 and IL-10 compared to the Saline and adjuvant group. Poly-T Leish/SM immunized mice exhibit a noteworthy reduction in the parasite burden (spleen and liver) through real-time PCR (96%) compared to the Saline group. Moreover, we observed higher nitrite secretion in antigen stimulated-culture supernatant. Spearman's *r* test analyses showed a strong negative correlation between splenic parasite burden and the percentage of T cells producing IFN-γ and TNF-α. We demonstrated that the Poly-T Leish/SM was potentially immunogenic, providing enhancement of protective immune mechanisms, and conferred protection reducing parasitism. Our candidate was considered potential against visceral leishmaniasis, and eventually, could be tested in phase I and II clinical trials in dogs. Acknowledgments: UFOP, PROPPI, CAPES, CNPq, and INCT-DT. **Supported by:**CNPq (MCTI/CNPq/CTBIOTEC nº 27/2013, 301526/2015-0, 486618/2013-7, 310104/2018-1 and 435224/2018-2); CAPES (Finance Code 001) **Keywords:**Visceral leishmaniasis;polyepitope vaccine;immunogenicity.

HP-27 - **INFECTION WITH *Leishmania (L.) amazonensis* IS AUGMENTED BY HOST SURFACE PROTEIN DISULFIDE ISOMERASE**

PAIVA, G.C.M.; STOLF, B.S..
USP, SÃO PAULO - SP - BRA.
E-mail: guilhermecarrara1@gmail.com

Leishmaniases comprise a spectrum of diseases caused by protozoan parasites of the genus *Leishmania* spp., transmitted by the bite of infected female phlebotomines. Infections by different *Leishmania* species may lead to tegumentary or visceral complications in humans. Chaperones present on macrophage surface play a fundamental role in the regulation of cellular homeostasis and may affect survival and infectivity of *Leishmania*. Protein disulfide isomerase (PDI) is one of the 20 most abundant chaperones of the endoplasmic reticulum (ER). The canonical role of PDI is to assist in the isomerization of disulfide bonds in nascent ER proteins, controlling the protein folding processes. The presence of PDI on the macrophage surface was associated with increased infection by *Leishmania (L.) chagasi*, a species associated with visceral leishmaniasis. The present study aimed to evaluate PDI role on the infection by *L. (L.) amazonensis*, a species responsible for cutaneous leishmaniasis. Flow cytometry was carried out to confirm the presence of PDI on macrophage surface. Bone marrow derived-macrophages (BMDM) were then blocked with anti-PDI polyclonal antibody and infected with promastigotes of *L. (L.) amazonensis*. BMDM from transgenic mice overexpressing PDI and wild type macrophages were also *in vitro* infected with *L. (L.) amazonensis*. *In vivo* imaging using M2269 *La*-LUC infection was done to compare lesion swelling and parasite load in transgenic and wild type mice. The results of flow cytometry indicated a low abundance of PDI on macrophage surface. Infection of macrophages blocked with anti-PDI was lower compared with infection in the presence of isotype antibody. Accordingly, infection of macrophages overexpressing PDI was higher than of the wild type counterparts. The *in vivo* results visually showed higher lesion swelling and parasite load in the PDI overexpressing group in comparison with the wild type. **Supported by:**(CAPES) 88887.360800/2019-00 **Keywords:** Leishmania (L;) amazonensis; PDI; Macrophage infection.

HP-28 - **The infection of mammalian cells and insect vectors with Trans-sialidase knockout Trypanosoma cruzi.**

BATISTA, M.F.[1]; CALDAS, G.D.A.B.[2]; ROCHA, F.D.S.[3]; SILVA, L.S.[3]; GUARNERI, A.A.[3]; BAHIA, D.[1]; TEIXEIRA, S.M.R.[1].
1. UFMG - MG - BRA; 2. CENTRO DE TECNOLOGIA DE VACINAS - CT VACINAS, MG - BRA; 3. INSTITUTO RENÉ RACHOU, FIOCRUZ, MG - BRA.
E-mail: batistamarinaf@outlook.com

Trans-sialidases (TS) are encoded by the largest gene family in the *Typanosoma cruz*i genome. Enzymatically active TS (aTS) correspond to 12 sequences. aTS are responsible for transferring sialic acid from host glycoconjugates to mucins present on the parasite surface. Using CRISPR/Cas9 technology, our group generated aTS knockout (aTSKO) parasites with undetectable levels of TS activity. Disruption of aTS genes did 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, aTSKO were unable to establish infection even in the highly susceptible IFN-g knockout mice. To investigate aTS roles, we performed *in vitro* infection assays with wild type (WT) and aTSKO parasites in two cell models: HeLa (non-phagocytic) and THP1 (phagocytic) cells. Our results confirmed that the lack of aTS did not impair parasite internalization and AMA multiplication in both cell types, but drastically affected TCT release. Given that several immune response genes, such as type I IFN, 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 and CXCL8. Contrarily to the aTSKO inability to infect mice, *in vivo* infection in *Rhodnius prolixus* insects was not affected by the lack of TS activity. After feeding fourth instar nymphs with blood containing WT and aTSKO epimastigotes no significant differences in total parasite numbers obtained from the digestive tract or in the urine were observed. We hypothesized that the infection success of *T. cruzi* in mammalian hosts are strictly dependent on the presence of aTS, thus aTSKO may serve as a target for vaccine designing in order to neutralize Chagas disease. **Supported by:** CNPq, CAPES **Keywords:**Active trans-sialidases (aTS);intracellular development;invertebrate infection.

HP-29 - *In vitro* and *in vivo* effects of zileuton against *Leishmania major*

ALVARENGA, V.B.S.; RABELO, R.A.N.; PEREIRA, R.D.D.; OLIVEIRA, N.F.D.M.; VAZ, L.G.; VIEIRA, L.Q.; MACHADO, F.S..
UFMG, MG - BRA.
E-mail: vivet21@yahoo.com.br

Cutaneous leishmaniasis is a public health problem causing a range of diseases from self-healing infections to chronic disfiguring disease. Currently, there is no vaccine for leishmaniasis, and drug therapy is often ineffective. 5-lipoxygenase (5-LO) is an enzyme required for production of leukotrienes and lipoxins and interferes with parasitic infections. Parasite as *Toxoplasma gondii* carries an enzyme like lipoxygenase activity that can interact with host biosynthetic circuits for endogenous negative signals that divert the host immune response and limite acute inflammation. Here, our aim was to evaluate the effect of zileuton (5-LO inhibitor) treatment during *Leishmania major* infection *in vivo*, and mainly to investigate if *L. major* itself wielding its own lipoxygenase. For this, promastigotes form of *L. major* was incubated with 5-LO inhibitor at 100, 33, 10, 3 and 1 micromolar or dimethyl sulfoxide (DMSO; used as a vehicle/solvent). *In vivo*, our preliminary data, demonstrated that treatment of *L. major*-infected C57BL/6 with zileuton reduced the parasite titer in the ear at 3 and 4 week after infection when compared with infected untreated mice. *In vitro*, zileuton induced a dose-dependent reduction of parasite numbers in an axenic culture. Collectively, these results suggest that zileuton treatment reduce the number of parasite *in vivo* and *in vitro* (axenic culture), mainly indicating that *L. major* promastigotes may carry a lipoxygenase-like activity that itself could respond to zileuton treatment. **Supported by:**: NPq, CAPES and FAPEMIG **Keywords:**L; major;Zileuton;5-lipoxygenase.

HP-30 - *Trypanosoma cruzi* induces DNA double-strand breaks and activation of the DNA damage response pathway in non-phagocytic cells

CÓRDOVA, R.A.G.[1]; DOS SANTOS, T.R.[1]; DE CASTRO, C.G.[2]; VIEIRA, J.A.[1]; SAKAMOTO-HOJO, E.T.[1]; BAQUI, M.M.A.[1].
1. FMRP / USP, RP - SP - BRA; 2. INSTITUTO BUTANTAN, RP - SP - BRA.
E-mail: raul.gonzales@usp.br

Intracellular pathogens such as viruses, bacteria, fungi, and protozoa often activate the DNA damage response (DDR) pathway in host cells. *Trypanosoma cruzi*, the etiological agent of Chagas disease, alters many signaling pathways in the host cells, including apoptosis inhibition, senescence induction, inflammatory response, and transcriptional alteration. Recently, we demonstrated that *T. cruzi* infection modulates the transcription and the splicing machinery in the host cell causing alterations in the nuclear compartments. We wondering whether these modulations could have been done in answer to the DNA damage caused by the presence of the parasite. The aim of this work was to investigate the existence of DNA damage and analyze the activation of the DNA damage response pathway in cells infected by *T. cruzi*. Our results showed that LLC-MK2 infected by *T. cruzi* analysed by comet assay present a high number of DNA breaks in host cells at 12hpi ($p<0.001$). On the other hand, maximal phosphorylation of H2AX is observed between 2 and 4 hpi analysed by immunofluorescence and western blotting ($p<0.001$). Throughout the infection process, γ-53BP1 and γ-ATM kinases remained active ($p<0.001$), while the γ-DNA-PKcs kinase had maximum activation at 12hpi ($p<0.001$). In contrast, γ-ATR and γ-Rad51 kinases were not altered during the evaluated period. Our data demonstrate that *T. cruzi* infection induces the activation of the DDR pathway in the host cell and shows the phosphorylation dynamics that the main proteins of the DDR pathway follow throughout the first 24 hours of infection. Our data also suggests that the breaks induced by *T. cruzi* infection are of the double-strand break (DSB) type and that the non-homologous end-joining (NHEJ) repair pathway may be activated. **Supported by:**FAPESP: 2018/03677-5; CAPES: Finance Code 001 (Project 88882.461733/2019-01); FAEPA / FMRP / USP **Keywords:**DNA damage response pathway;Trypanosoma cruzi infection;host cell nucleus.

HP-31 - **EVIDENCE OF ANEUPLOIDY IN *TRYPANOSOMA CRUZI* BY FISH (FLUORESCENCE *IN SITU* HYBRIDIZATION) USING SPLICE LEADER GENE AS SPECIFIC DNA PROBE**

PAUER, A.B.[1]; ASANUMA, C.M.Y.[1]; TEIXEIRA, T.L.[1]; TOQUEIRO, C.M.O.[1]; FERREIRA, E.R.[1]; CALDERANO, S.G.[2]; SABBAGA, M.C.Q.B.E.[2]; MORTARA, R.[1]; DA SILVEIRA FILHO, J.F.[1].
1. UNIFESP, SP - BRA; 2. INSTITUTO BUTANTAN, SP - BRA.
E-mail: agustinapauer@hotmail.com

T. cruzi presents a high degree of intraspecific genetic variability, with possible implications for its adaptation in different hosts. Several studies have shown aneuploidy in *T. cruzi*, even though it´s considered a diploid organism. Whole genomic approaches revealed chromosomal ploidy within a same *T. cruzi* population. Here we identified aneuploid events in *T. cruzi* by FISH that allows the evaluation of ploidy at the individual cell level. We used the Spliced Leader sequence (SL) as a chromosome-specific marker. SL gene encodes an essential RNA for translation, which is transcribed from a tandemly arranged repeated sequence. Epimastigotes (CL Brener) were grown in axenic cultures and cells that bear more than one flagellum were disregarded, excluding parasites in mitosis. Ploidy was estimated from 600 labeled cells for each probe by epifluorescence microscopy, by 3 independent observers in biological replicates. Ploidy frequency was as follows, 77.7% of cells were monosomic, 16.3% disomic, 1.8% trisomic and 0.5% polysomic. No statistically significant differences were found between the counts ($p<0.05$). Aneuploidy events were validated after cell cycle synchronisation by hydroxyurea (HU) by confocal microscopy. To investigate if there's a difference in the ploidy during cell cycle, epimastigotes were treated with HU for 18 h and samples collected in G1 (2h) and G2 (14h) for flow cytometry and FISH analyses. No statistically significant differences were found between treated and control groups, confirming the aneuploidy within the population. The high proportion of monosomic cells could be explained by a mistake in chromosomal segregation due to nondisjunction of sister chromatids during mitosis. A potential consequence is the change in the relative dosage of products from genes located on the missegregated chromosomes. To confirm the occurrence of chromosome aneuploidy, we will test markers (single copy genes) from the same homologue where the SL genes were mapped. **Supported by:** FAPESP 2016/15000-4, CAPES, CNPq **Keywords:** TRYPANOSOMA CRUZI;FLUORESCENCE IN SITU HYBRIDIZATION; ANEUPLOIDY.

HP-32 - **CHARACTERIZATION OF *Leishmania (L.) amazonensis* OLIGOPEPTIDASE B AND ITS ROLE IN MACROPHAGE INFECTION**

BARBOSA, G.R.; MARANA, S.R.; STOLF, B.S..
USP, SP - BRA.
E-mail: gustavorolim@hotmail.com

Leishmania spp. are parasitic protozoa that cause leishmaniasis, a disease endemic in 98 countries. *Leishmania* promastigotes are transmitted by the vector and differentiate into amastigotes within phagocytic cells of the vertebrate host. To survive in multiple and hostile environments, the parasite has several virulence factors. Oligopeptidase B (OPB) is a serine peptidase present in prokaryotes, some eukaryotes and some higher plants. It has been considered a virulence factor in trypanosomatids, but only a few studies, performed with Old World species, analyzed its role in *Leishmania* virulence or infectivity. *L. (L.) amazonensis* is an important agent of cutaneous leishmaniasis in Brazil. The *L. (L.) amazonensis* OPB encoding gene has been sequenced and analyzed in silico but has never been expressed. In this work, we produced recombinant *L. (L.) amazonensis* OPB and showed that its pH preferences, Km and inhibition patterns are similar to those reported for *L. (L.) major* and *L. (L.) donovani* OPBs. Since *Leishmania* is known to secrete OPB, we performed in vitro infection assays using the recombinant enzyme. Our results showed that active OPB increased in vitro infection by *L. (L.) amazonensis* when present before and throughout infection. Our findings suggest that OPB is relevant to *L. (L.) amazonensis* infection, and that potential drugs acting through OPB will probably be effective for Old and New World Leishmania species. OPB inhibitors may eventually be explored for leishmaniasis chemotherapy. **Supported by:**FAPESP (Processo: 2019/02391-3) / CAPES **Keywords:**L; (L;) amazonensis;Oligopeptidase B;Macrophage infection.

HP-33 - **Study of the regulation of iron and heme transporters in *Leishmania (L.) amazonensis***

HONG, A.A.[1]; BOY, R.L.[1]; DE AQUINO, G.P.[1]; GOES, W.M.[2]; TEIXEIRA, S.M.R.[2]; FLOETER-WINTER, L.[1]; LARANJEIRA-SILVA, M.F.[1].
1. INSTITUTO DE BIOCIÊNCIAS -USP, SP- BRA; 2. INSTITUTO DE CIÊNCIAS BIOLÓGICAS -UFMG, MG - BRA.
E-mail: avery.ahyun.hong@ib.usp.br

Leishmaniases, a spectrum of diseases caused by protozoan parasites of the genus *Leishmania*, affects millions of people around the world. During infection, nutrient availability within the phagolysosome is known to have significant effects on parasite replication and virulence. These processes require the acquisition of essential nutrients such as iron and heme from the host since *Leishmania* does not have iron storage proteins and a complete heme biosynthesis pathway. *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. Identification of several proteins that participate in the transport of iron and heme was crucial for understanding these metabolic pathways in *Leishmania*, and the loss of those transporters causes serious defects in the differentiation and/or multiplication of these parasites in the host. Hence, our goal is to investigate the cross-regulation between iron and heme transporters of *Leishmania*. Thereupon, we will evaluate parasites genetically modified to overexpress LIT1 in LHR1 knockout or LHR1 in LIT1 knockout parasites. During the validation of previously obtained LIT1 knockout mutants via homologous recombination, we discovered that LIT1´s chromosome (30) is tetrasomic in *L. amazonensis* PH8 strain. Therefore, we are generating LIT1 knockout mutants using the CRISPR/Cas9 genome editing approach. In parallel, we obtained parasites overexpressing LIT1 tagged with GFP in the LHR1 single knockout background. These mutant parasites are being evaluated regarding replication and expression of genes and proteins essential for iron and heme metabolism. Characterization of the regulation of the pathways related to iron and heme transport is critical for a better understanding of *Leishmania* physiology and the host-pathogen interaction.
Supported by:2021/03355-0 **Keywords:**Leishmaniasis;Transition Metals;Host-Parasite Interaction.

HP-34 - **First isolation, genomic and biological characterization of a *N. caninum* strain isolated from free-ranging chicken**

SILVA, M.F.; DA COSTA, M.S.; MOTA, C.M.; TORRES, J.D.A.; FERREIRA, F.B.; MINEO, J.; MINEO, T.W.P..
UNIVERSIDADE FEDERAL DE UBERLÂNDIA, - MG - BRA.
E-mail: tiago.mineo@ufu.br

Neospora caninum induces abortions in cattle and consequent economic impact, since there are no treatments nor vaccines against the disease. It is known that birds are reservoirs for *N. caninum*, but the actual relevance of neosporosis in birds is not clear. In the present work we aimed to identify whether free-range chickens would be natural hosts of *N. caninum*. First, we analysed whether chickens sampled in Brazil had specific antibodies against the parasite. We then performed bioassays in mice with brain samples of positive animals. That led us to isolate a new strain of *N. caninum* (Nc-UDI4), with identity confirmed by positive parasite-specific Nc5 genomic sequence, further confirmed by the detection of IgG antibodies in the serum of infected mice. NGS of Nc-UDI4 showed coverage of 87.6% of the reference genome (Nc-Liv) and a series of SNPs and InDels in genes related to adhesion, invasion and survival of the parasite. Accordingly, Nc-UDI4 had lower invasion/replication rates compared to Nc-Liv. Microsatellite analysis showed that Nc-UDI4 is closely related to the genotypes found in Argentinian strains and Nc-1, and relatively distant from Nc-Liv. Also, we observed that that Nc-UDI4 and Nc-1 are less virulent than Nc-Liv in infected C57BL/6 mice, due to higher survival rates, reduced parasite burden and inflammation and a more prominent Th1-biased cytokine profile. In conclusion, we determined that chickens may naturally host *N. caninum*, and the strain we were able to isolate had common features to other known strains of the parasite. **Supported by:**CNPq 313761/2020-5; FAPEMIG PPM-00547-17, APQ-01313-14, REDE-00313-16 **Keywords:**Neospora caninum;Chicken;Isolation.

HP-35 - **Influence of multiple inoculum of *L. (L.) infantum* promastigotes in an experimental model in BALB/c mice**

DE FREITAS, F.R.N.; DE CARVALHO, C.A.; DE ANDRADE JR, H.F..
FACULDADE DE MEDICINA DA USP, SP - BRA.
E-mail: flaviafreitas@usp.br

Visceral leishmaniasis (VL) is endemic in Brazil and affects 3,500 individuals/year, with a proportion of 1 patient for 18 infected. It is caused by the parasite *Leishmania (Leishmania) infantum* and transmitted by sandflies *Lutzomyia longipalpis* species. Mice are usually resistant to infection, similar to human self-limiting disease. In experimental models, a single inoculum is used to promote infection. However, for canine reservoirs, the host may receive multiple infective doses of different sand flies, and it can affect the development of the disease. To clarify this aspect, we intend to simulate multiple inocula in a mice model and relate it to the progression of the disease. BALB/c mice were infected intraperitoneally with 1 inoculum (Group 1) or with 4 weekly inoculums (Group 2) of $1X10^7$ promastigotes. The infection was monitored by measuring the production of IgG antibodies, detected by conventional ELISA (ELISAc) and dissociative ELISA (ELISAd). The animals were euthanized at 30 days of infection, and spleen and liver fragments were removed to determine the parasite load by real-time PCR, and paraffin embedding for conventional HE histology and immunohistochemistry. For the parasite load, the protein concentration of the organ homogenate was expressed as the number of parasites/mg of protein. Statistical analysis was performed using the Kruskal Wallis method with Dunn's post-test. The experimental group 1 showed increasing levels of IgG antibodies and the presence of circulating monovalent immune complexes (CIC) in the last post-infection periods (21st and 28th days). In group 1, the mean parasite load in the spleen was $2X10^5$ and in the liver $2X10^4$ parasites/mg of protein, higher than in group 2, mean of $1X10^4$ in the spleen ($p<0.01$) and $4X10^3$ in the liver ($p<0.001$). With this study, we observed that qPCR showed higher parasite load in animals that received a single dose. In addition, that the challenge with multiple inocula can protect the host from disease. **Keywords:**Experimental Infection;BALB/c mice;Visceral Leishmaniasis.

HP-36 - **Characterization of extracellular vesicles released by neutrophils stimulated with Leishmania amazonensis**

WACLAWIAK, I.; RAMOS, G.D.S.; WERNECH, M.V.I.; COSTA, A.G.B..
UFRJ- RJ - BRA.
E-mail: waclawiak@msn.com

Neutrophils are the most abundant leukocytes in the blood and play an extremely important role in the inflammatory process and the body's defense against pathogens. In the context of *Leishmania* infection, neutrophils are the first cells to migrate to the affected tissue, where they can either promote the control of the parasite or exacerbate the infection, as reported in the literature. In addition to their microbicidal mechanisms, neutrophils also interact with macrophages, the parasite's final host cells. In response to different inflammatory stimuli, neutrophils release extracellular vesicles (EVs), which are capable of regulating numerous physiological processes and contribute to tissue homeostasis or propagation of infectious agents. These particles have immunomodulatory properties and potentiate neutrophil migration. The participation of neutrophil EVs during *Leishmania amazonensis* (L.a) infection has not been explored. Our aim in this work is to characterize EVs released by neutrophils stimulated by this pathogen. For that, human neutrophils were isolated from peripheral blood of healthy individuals and stimulated or not with *L. amazonensis* promastigotes for 1h at 37ºC. To obtain the fractions enriched in exosomes and microvesicles, we chose to use ultracentrifugation, a widely technique used for vesicle purification. Our preliminary results indicate that neutrophils stimulated with parasites release more exosomes and fewer microvesicles than non-stimulated neutrophils, as observed by nanoparticle tracking analysis. Stimulation with L.a did not affect the size distribution profile of the particles. EVs showed spherical and membrane-delimited morphologies, as observed by negative contrast transmission electron microscopy. We are currently characterizing the content of these vesicles and performing functional experiments to understand how these microparticles can influence the macrophage response and infection by the parasite. **Supported by:**CAPES/FAPERJ/CNPq **Keywords:**Extracellular Vesicles;Neutrophils;Leishmania amazonensis.

HP-37 - **Sand fly yellow salivary proteins modulate neutrophil response to Leishmania parasites**

DA SILVA, J.O.[1]; WACLAWIAK, I.[1]; VALENZUELA, J.G.[2]; OLIVEIRA, L.F.B.[2]; COSTA, A.G.B.[1]. 1. UFRJ- RJ - BRA; 2. NIH, USA.
E-mail: jullyannaoliveira@yahoo.com.br

Leishmaniasis is a group of neglected diseases caused by parasites of the genus *Leishmania,* which are transmitted by sandfly bites. Factors derived from both the insect and the protozoan, such as microbiota, saliva and proteophosphoglycans, are inoculated into the skin of the vertebrate host at the time of the bite and can modulate the immune response in favor of the infection. After the sand fly bite, circulating neutrophils are the first cells recruited to the site of infection and the first cells to be infected. Our group recently reported that proteins from the insect's saliva, the yellow family of proteins, act as chemoattractants for neutrophils as well as exacerbate the infection *in vivo*. In this study, we investigated the role of these proteins in the infection of human neutrophils with *Leishmania* parasites and consequences that arise from this initial interaction *in vitro*. Our results show that yellow proteins modulate the responses of infected neutrophils. Treatment of neutrophils infected by *Leishmania major* and *Leishmania infantum* with yellow proteins induced an increase in parasite survival, reduction of NETs release and a reduction in the percentage of neutrophils in direct contact with the parasite. *Leishmania major*-infected neutrophils treated with yellow proteins showed a higher elastase release. Furthermore, cultures of macrophages infected with *L. major* and treated with supernatant of yellow protein-treated neutrophils showed an increase in parasite survival. Our data reveal an immunomodulatory role for the yellow family of proteins on infected neutrophils and indicate their possible relevance on human infection. **Supported by:**CNPq, FAPERJ, CAPES **Keywords:**Neutrophils;Leishmania;yellow proteins.

HP-38 - **Identification of potential Leishmania infantum sirtuin inhibitors**

REIS, A.H.[1]; DA SILVA, G.B.[1]; CUNHA, P.D.R.B.B.[2]; MORAES, C.B.[2]; FREITAS JUNIOR, L.H.[2]; ROTILI, D.[3]; MAI, A.[3]; MORETTI, N.[1].
1. UNIFESP, SP - BRA; 2. USP, SP - BRA; 3. UNIROMA, ITA
E-mail: artur77reis@gmail.com

Different species of Leishmania cause Leishmaniasis in 90 countries. It has a clinical spectrum ranging from cutaneous and mucocutaneous to visceral lesions. Its cycle life shifts between invertebrate and vertebrate hosts, facing different environmental changes, which require rapid adaptations to survival. Post-translational modifications, such as acetylation, has been implicated in the regulation of diverse cellular processes in eukaryotic organisms. We described the acetylated proteins of procyclic, metacyclic and amastigote forms of L. mexicana and found a differential acetylation profile among them, suggesting that might be important for parasite differentiation. Protein's acetylation are regulated by two families of enzymes: lysine acetyltransferases and lysine deacetylases (KDACs). Among the KDACs, sirtuins are involved in the regulation of several biological processes and have been explored as drug targets in different human diseases. Considering the importance of protein acetylation in Leishmania, this work explored the potential of sirtuins from L. infantum as drug target for the identification of inhibitors for future treatment. We screened a library of 80 sirtuin inhibitors using in vitro infection assays to identify molecules with inhibitory activity, three were chosen for further biochemical validation. These assays focused on testing the inhibitory deacetylation activity of the recombinant L. infantum sirtuins. The 3 genes (LiSir2rp1-3) were cloned in pNIC28 vector, sequenced and transformed into bacteria for heterologous expression. The expression of these proteins were confirmed and established the purification protocol for LiSir2rp1 and rp3. We confirmed the in vitro deacetylation activity of the enzyme LiSir2rp3 by biochemical characterization assays. One of 3 previously chosen inhibitors against LiSir2rp3 performed inhibitory activity greater than 30%. New experiments will test these inhibitors against LiSir2rp1-2. **Supported by:**FAPESP 2020/14754-0 **Keywords:**Leishmania;sirtuins;inhibitors.

HP-40 - **Characterization of manganese transport mediated by the iron transporter LIR1 in *Leishmania***

DE AQUINO, G.P.; HONG, A.A.; FLOETER-WINTER, L.; LARANJEIRA-SILVA, M.F..
INSTITUTO DE BIOCIÊNCIAS, SP - BRA.
E-mail: giovana.parreira@usp.br

Leishmaniases are a group of diseases caused by protozoan parasites of the genus *Leishmania*. These parasites alternate between insect and mammalian hosts, going through dramatic changes with shifts in temperature, pH, and availability of nutrients. Among these nutrients, iron is an important cofactor of several enzymes, but can also be highly toxic when free and at high concentrations in the cytoplasm. *Leishmania Iron Regulator 1* (*LIR1*) was identified and characterized in *Leishmania* as a plasma membrane transporter essential for iron efflux and regulation of the intracellular concentration of iron and other transition metals, such as manganese. LIR1 deficiency enhances the toxic effect of excess iron and manganese during promastigote replication. Like iron, manganese is also a cofactor of several *Leishmania* enzymes, in particular arginase, which is essential for parasite replication and infection establishment in mammalian hosts. Considering the significance of manganese for *Leishmania* and the evidence that LIR1 also modulates the intracellular levels of this metal, our main goal is to characterize the manganese transport mediated by LIR1 in *L. amazonensis*. Inductively coupled plasma mass spectrometry (ICP-MS) analysis revealed that LIR1 prevents the accumulation of manganese in *Leishmania.* Moreover, *Leishmania*'s arginase expression and enzymatic activity is modulated by LIR1 expression. In parallel, we observed that promastigotes overexpressing LIR1 present a delayed *in vitro* growth, which was also exacerbated by manganese supplementation. However, manganese intracellular accumulation was not affected by LIR1 overexpression. We anticipate that further ICP-MS analysis of manganese transport kinetics in these mutants will uncover the direct involvement of LIR1 in the transport of manganese across promastigotes' plasma membrane. **Supported by:**FAPESP - 2019/09715-9 **Keywords:**Leishmania;Manganese;Arginase.

HP-41 - **Removal of Complement Membrane Attack Complex (MAC) Pores by Plasma Membrane Repair: an ancestral mechanism of Eukaryotes enabling pathogen immune evasion.**

BARROS, L.V.R.[1]; REGINALDO, M.C.[1]; MOREIRA, A.L.D.S.[2]; DUARTE, V.F.D.A.[2]; DE OLIVEIRA, T.Q.[2]; HORTA, M.D.F.M.[2]; GONTIJO, N.D.F.[1]; GOMES, T.D.C.[2].
1. UFMG, MG - BRA; 2. UNIVERSIDADE FEDERAL DE GERAIS, MG - BRA.
E-mail: lvriosb@gmail.com

Plasma membrane repair (PMR) is a crucial physiological mechanism of eukaryotic cells that promotes the resealing of damages eventually made on 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 are able to 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, respectivelly. By using conventional, confocal and live imaging microscopies, immunobiochemical methods and flow cytometry 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, similar to mammalian cells, *L.amazonensis* promastigotes are able to 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 provide the parasites with 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:**Plasma Membrane Repair;Complement System;Immune Evasion.

HP-42 - **Genomic Analysis of Leishmaniinae Parasites Isolated from Human Visceral Leishmaniasis**

TORRES, C.[1]; ROGERIO, L.[1]; TAKAMIYA, N.T.[1]; TAKAHASHI, T.Y.[1]; DE ALMEIDA, R.P.[2]; SILVA, J.S.[3]; JEFFARES, D.C.[4]; MARUYAMA, S.R.C.[1].
1. UNIVERSIDADE FEDERAL DE SÃO CARLOS, SP - BRA; 2. UNIVERSIDADE FEDERAL DE SERGIPE, ARACAJU - SE - BRA; 3. USP, RP - SP - BRA; 4. UNIVERSITY OF YORK, ENG
E-mail: carolinetorres@estudante.ufscar.br

Leishmaniases are a group of neglected diseases caused by a variety of Leishmania species. In Brazil, Visceral Leishmaniasis (VL) is caused by L. infantum transmitted by sandfly vectors. It is the most severe form of the disease. Recently, we identified clinical isolates from VL cases that do not belong to the Leishmania genus and phylogenetic analysis showed a close relationship with Crithidia fasciculata, a monoxenous trypanosomatid considered non-pathogenic to humans. We called them Crithidia-like. Here, we characterized 47 clinical isolates from VL patients (Sergipe, Brazil) typed as Crithidia-like or L. infantum using an in-house PCR/qPCR assay. Whole-Genome Sequencing (WGS) of these samples were performed in Illumina platform (150 bp paired-end reads, average of 2 GB per isolate) and analyzed using bioinformatic pipelines in Comparative Genomics. Forty two isolates were identified as Crithidia-like and 5 as L. infantum. WGS reads were mapped to Crithidia-like LVH60aC1 assembly (36 pseudochromosomes/contigs). Genetic variations among them were analyzed. Overall, the coverage depth by pseudochromosomes was 40X. Somy values were characterized based on the median of median read depth coverage of all pseudochromosomes, calculated by the SAMtools program and visualized by ggplot2 package on the R studio program. Pseudochromosomes 12, 20 were classified as trisomic and pseudochromosome 36 as tetrasomic. Variant calling was done by SnpEff program and detected an average of 410.000 per isolate in which approximately 276.500 are single nucleotide polymorphisms (SNP) and 45.585 are insertions or deletions (INDELS). Roughly 30,5% of the variants are missense (causes a change on codon and produces a different amino acid), 0,3% are nonsense (causes a change on codon and and results in a stop codon) and 69,2% are silent (causes a change on codon but produces the same amino acid). Further, copy number variations (CNV) and mitochondrial DNA content (kDNA) will be analyzed. **Supported by:**FAPESP 2016/20258-0 **Keywords:**Visceral Leishmaniasis;Whole-Genome Sequencing;Crithidia-like.

HP-43 - **Clinical and parasitological aspects of the treatment of canine visceral leishmaniasis with miltefosine and associations in Florianópolis, Santa Catarina State, Brazil**

ROSAR, A.D.S.; MARTINS, C.L.; GRISARD, E.C.; STEINDEL, M.; QUARESMA, P.F.; STOCO, P.H..
UNIVERSIDADE FEDERAL DE SANTA CATARINA, FLORIANÓPOLIS - SC - BRA.
E-mail: carol4952@hotmail.com

Leishmaniases are zoonotic infectious diseases caused by protozoan parasites of the genus *Leishmania*. Visceral Leishmaniasis (VL) caused by *Leishmania infantum* is the most serious form of the disease, having the domestic dogs as the main reservoirs in the urban transmission cycle. Canine visceral leishmaniasis (CVL) was reported in southern Brazil in 2008, following the occurrence of human cases. In Florianópolis (SC State), CVL was reported in 2010, following the detection of human cases in 2017. Miltefosine has been used for CVL treatment since 2016 in Brazil, but few studies investigated the effectiveness of the treatment of naturally infected dogs. We have assessed clinical, parasitological and histopathological aspects of dogs naturally infected with *L. infantum*, before and after the treatment with miltefosine and associations. Skin and lymph node samples were collected from 24 dogs from Florianópolis before (T0) and after treatment, as follows: 31 days after the first dose of miltefosine (T1) and 6 months after the end of treatment (T2). A significant decrease ($p<0.05$) in the average parasite load on the skin was detected by qPCR on T1, along with a general clinical improvement. Linear regression analysis revealed a positive correlation between the reduction of the parasite load, the clinical improvement of the animal, and the reduction of the inflammatory infiltrates on the skin. In addition, the parasite skin load of dogs submitted to therapeutic regimen 1 (miltefosine+allopurinol+domperidone) was significantly lower ($p<0.05$) than animals treated with regimen 2 (miltefosine or miltefosine+allopurinol or miltefosine+immunotherapy). These results indicate that, despite distinct drugs used in association, the treatment of CVL based on miltefosine contributed to the reduction of the parasite load in the skin and the overall clinical improvement of the animals, but not leading to parasitological cure. **Supported by:**CAPES, FAPESC, FINEP, CNPq **Keywords:**Leishmania infantum;public health;Canine Visceral Leishmaniasis.

HP-44 - **Intracellular views of *Toxoplasma gondii* in cleaved cells by HR-SEM**

SILVA, T.A.; ATTIAS, M..
INSTITUTO DE BIOFÍSICA CARLOS CHAGAS FILHO, RIO DE JANEIRO - RJ - BRA.
E-mail: tatibio86@gmail.com

Toxoplasma gondii, an obligate intracellular protozoan, infects up to one-third of the world population. Although most infections are asymptomatic, this protozoan may cause retinal lesions and, in immunocompromised individuals or when contracted congenitally, can lead to life-threatening infections involving the central nervous system. *T. gondii* has evolved several strategies for successful invasion and intracellular survival and multiplication. It includes the apical complex, composed of specialized secretory organelles, namely micronemes and rhoptries, and cytoskeletal elements, such as the polar rings and the conoid. Electron microscopy is a powerful tool to study many aspects of *T. gondii* development inside host cells. For many years, intracellular *T. gondii* could not be observed by Scanning Electron Microscopy (SEM). Field Emission Scanning Electron Microscopy enhanced the resolution power of SEM to a level comparable to TEM. We have applied the method proposed by in 1986 [1] to expose the cytoplasm and organelles of free cells to observe LLC-MK2 cells infected with the RH strain of *T. gondii* with the High-resolution SEM Auriga 40-ZEISS. The method consists in packing free cells infected with *T. gondii* together in a matrix of gelatin and chitosan, freezing and cleaving the assembly. With this method, we obtained a large number of cleaved host cells with parasites inside, so aspects of both the host cell and the parasite could be observed. Rhoptries, micronemes, the tubules of the intravacuolar network, aspects of parasites undergoing endodiogeny and the cytoskeleton around the parasitophorous vacuole could be observed. We believe that this is a simple and reliable method that could be applied not only to *T. gondii*, but other intracellular parasites to reveal new features of their biology. **Supported by:**Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro - E-26/200.135/2020 **Keywords:**Cell Ultrastructure;Scanning Electronic Microscope ;Fracturing method.

HP-45 - **Role of SAPA repeats present in Trans sialidases during *Trypanosoma cruzi* infection**

JÚNIOR, C.R.D.A.[1]; DOS SANTOS, N.S.A.[1]; OTTINO, J.[1]; DE CASTRO, J.T.[2]; SANCHES, R.C.D.O.[1]; OLIVEIRA, S.C.[1]; GAZZINELLI, R.T.[3]; TEIXEIRA, S.M.R.[4].
1. UFMG, MG - BRA; 2. FIOCRUZ MG, MG - BRA; 3. FIOCRUZ MG/UFMG/CT-VACINAS, - MG - BRA; 4. UFMG/CT-VACINAS, MG - BRA.
E-mail: carlos.almeidajr7@gmail.com

Proteins with amino acid repeats have been identified in different protozoa. Among proteins that contain repetitive amino acid sequences in *Trypanosoma cruzi*, are trans-sialidases (TS), which are responsible for transferring sialic acid from host glycoconjugates to mucins on the parasite surface. TS activity is part of a mechanism related to the parasite evasion of the host imune system. Present in more than 1000 copies in the parasite genome, only 16 TS sequences encode proteins with catalytic activity and these proteins have a C-terminal domain containing variable numbers of amino acids repeats known as SAPA (shed acute parasite antigen). In addition, SAPA domain increases the stability of the enzyme in the bloodstream, suggesting that it may contribute to the parasite virulence. To investigate the role of SAPA repeats and their influence on the host immune system, three recombinant versions of an active TS were produced: full-length protein, TS without repeats, and only SAPA repeats. BALB/c mice were immunized with proteins and then challenged with a virulent strain of *T. cruzi*. Both the full-length protein and the protein without the SAPA domain protected animals after challenge but the protein without SAPA provided better protection. Importantly, immunization with the portion containing only SAPA repeats exacerbated the infection. ELISA assays showed that all proteins induced IgG production and its subclasses and that the antibodies induced by the full-length TS protein were directed to the SAPA repeats indicating its immunodominance. Analysis of the cellular response showed higher levels of IFN-γ produced by splenocytes from animals immunized with the TS protein without SAPA repeats, compared to splenocytes from mice immunized with the other two antigens. Taken together these results indicated that immunization with TS antigen without SAPA repeats induces the development of a protective Th1 response, essential for intracellular pathogen infection control. **Supported by:**Capes **Keywords:**Trans-sialidase (TS);SAPA repeats;T; cruzi.

HP-46 - **Identification and characterization of iron transporters candidates in Leishmania amazonensis glycosomes**

BOY, R.L.; AOKI, J.; ZAMPIERI, R.A.; FLOETER-WINTER, L.
. INSTITUTE OF BIOSCIENCES, UNIVERSITY OF SÃO PAULO, SP - BRA.
E-mail: rlopesboy@usp.br

The protozoan parasites of the genus *Leishmania* are responsible for the diseases 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. The identification and study of parasite genes involved in iron metabolism and transport revealed that the availability of iron plays a central role in virulence. 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 encoding proteins addressed to the glycosome, which are trypanosomatids unique organelles. Therefore, our goal is to identify and characterize genes involved in the transport of iron into the *Leishmania* glycosome. *In silico* analysis of the *Leishmania* transcripts modulated by iron deprivation revealed 11 putative genes containing predicted glycosomal addressing signals (PTS1 or PTS2) and transmembrane domains. By immunofluorescence, we confirmed the glycosomal promastigotes compartmentalization of the proteins encoded by 2 of these genes. Moreover, the overexpression of 5 of these genes impacted promastigotes *in vitro* growth. Further characterization of these overexpressors and knockout parasite strains will contribute to elucidate the mechanisms involved in the metabolism and transport of iron into the *Leishmania* glycosome and may indicate new targets for the development of chemotherapeutic agents for the treatment of leishmaniases. **Supported by:**FAPESP 2017/23933-3 **Keywords:**Transfection; Immunofluorescence ; Gene Expression.

HP-47 - **EVALUATION OF SPECIES-SPECIFIC GENES OF Leishmania infantum AS POTENTIAL TARGETS FOR DIAGNOSIS OF VISCERAL LEISHMANIASIS**

STEFFLER, J.M.D.[1]; ROGERIO, L.[1]; TAKAMIYA, N.T.[1]; DE CORREIA, C.R.S.T.B.[2]; TEIXEIRA, F.R.[1]; MARUYAMA, S.R.C.[1].
1. DEPARTAMENTO DE GENÉTICA E EVOLUÇÃO UNIVERSIDADE FEDERAL DE SÃO CARLOS - DGE/UFSCAR, SP - BRA; 2. USP, SÃO CARLOS - SP - BRA.
E-mail: joselmajoselma@gmail.com

Leishmaniasis is a public health problem found in many countries and caused by several *Leishmania* species. Efficient and fast diagnosis is essential to assure the effectiveness of the treatment, thus, the search for new antigens capable of stimulating the immune response will help to develop more accurate serological tests for diagnosis. Our aim was searching for species-specific genes of *L. infantum* through *in silico* analyses of *Leishmania* genomes using Tritryp database by the following criteria: i) Orthology (excluding other Leishmania species and other trypanosomatids); ii) genes encoding proteins; iii) presence of signal peptide and/or transmembrane domain; iv) absence of post-translational modification. *L. infantum* candidate genes were analysed with Bepipred tool (threshold: 0.500) to predict B cell epitopes. Codon analysis was performed with GenScript Rare Codon Analysis Tool to assess the viability of heterologous recombinant protein expression. Genomic DNA extraction was performed using the Kit Wizard® SV Genomic DNA Purification System (Promega). Primers was designed using Ape and SnapGene programs and analysed with OligoAnalyzerTM tool. PCR reactions were performed and the analysis of the products was performed by agarose gel electrophoresis. A total of 24 genes was found in TriTryp search. Where, 17 have a signal peptide, 4 have a transmembrane domain and 3 have both. In *in silico* analysis we selected six genes, from chromosomes 7, 15, 18, 22 and two from 31. They presented average values of prediction of linear epitopes of B cells in 0.521 and 0.619. Codon usage analysis of these genes showed an adequate codon frequency and adaptability (codon adaptation index in 0.63 and 0.73), with the percentage of low frequency codons ranging between 8% to 18%, indicating a viable expression in *E. coli*. Out of six selected genes, three were successfully amplified by PCR using gDNA from *L. infantum* as template and will be used to perform cloning in experiments. **Supported by:**CAPES: 88887.486526/2020-00 / FAPESP: 2016/20258-0 **Keywords:**Visceral leishmaniasis; Diagnosis; bioinformatics.

HP-48 - **Dual RNA-seq mapping in visceral leishmaniasis: dataset of parasite transcripts in human blood transcriptome upon Leishmania infantum infection.**

ROGERIO, L.A.[1]; ALVES, E.G.[1]; TAKAMIYA, N.T.[1]; TORRES, C.[1]; DE ALMEIDA, R.P.[2]; SILVA, J.S.[3]; MARUYAMA, S.R.C.[1].
1. UFSCAR, SP - BRA; 2. UFS, SE - BRA; 3. FIOCRUZ, SP - BRA.
E-mail: elgmsalvs.bio@gmail.com

Visceral Leishmaniasis (VL) is a neglected disease caused by Leishmania infantum parasites in Brazil and can be lethal if untreated. Blood transcriptome of infected patients is valuable to reveal molecular mechanisms associated to development of the disease. When transcriptome of host is obtained through RNA-seq, the gene expression of parasite can also be ascertained. Here, our aim was determining a survey of parasite transcripts in blood transcriptome data of patients using dual RNA-seq mapping strategy. For this, fastq files were trimmed using Trimmomatic and high quality reads of groups A (asymptomatic individuals), PD0 (patient with active infection, before treatment), PD180 (cured patients, six months after treatment) from RNA-seq experiment E-MTAB-11047 (Maruyama et al. 2022) were aligned to the concatenated genome (parasite + host) built with Leishmania infantum (GCA_900500625.1) and Homo sapiens GRCh38.p13 genomes obtained from ENSEMBL database, using STAR aligner. A read count table was retrieved for each RNA-seq sample. Although parasitemia is not a feature of L. infantum infection, we were able to identify reads from parasites in blood samples of the three groups, being the PD0 samples those with highest number of representative transcripts totaling 525 mapped genes across the group with an average of 103 mapped genes per sample. For PD180 and A group, an average of 33 and 30 genes were mapped per sample, respectively. Gene ID searches on TriTrypDB revealed that most genes coding for hypothetical conserved proteins, amino acid metabolism and 40S ribosomal protein synthesis. Functional annotation of L. infantum mapped genes are being done on TriTrypDB to identify related biological pathways. Interestingly, parasite transcripts were detectable in cured patients and asymptomatic individuals, suggesting parasite persistence scenario even with absence of clinical signs of VL. **Supported by:**Fundação de Amparo à Pesquisa do Estado de São Paulo FAPESP - JP 2016/20258-0; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); FAPESP/ TT3 - 2022/01525-9 **Keywords:**Visceral leishmaniasis;RNA-seq;dual transcriptome mapping.

HP-49 - **THE INHIBITOR OF SERINE PEPTIDASES, ISP2, OF *Trypanosoma cruzi* PREVENTS TMPRSS2-MEDIATED PAR2-TLR4 CROSSTALK AND MODULATES INFECTION AND INFLAMMATION**

COSTA, T.F.R.[1]; GOUNDRY, A.L.[1]; DE CARVALHO, D.B.[1]; RODRIGUES, N.S.[1]; DE ABREU, M.F.[1]; DOS REIS, F.C.G.[2]; LIMA, A.P.C.D.A.[1].
1. INSTITUTO DE BIOFISICA CARLOS CHAGAS FILHO, UFRJ- RJ - BRA; 2. FUNDAÇÃO OSWALDO CRUZ, - PR - BRA
E-mail: tcosta@biof.ufrj.br

Ecotin is a potent inhibitor of family S1A serine peptidases (SPs) and was first isolated from the periplasm of *E. coli*. Ecotin-like inhibitors were identified in Trypanosomatids and have been denominated **I**nhibitor of **S**erine **P**eptidase (ISP). Trypanosomatids apparently lack SPs from S1A family. In *L. major*, ISP2 is required for parasites successful infection since prevents the activation of the TLR4-NE pathway in macrophages and was also associated with reduction of iNOS expressing monocytes in lesions in mice. Here, we investigated the role of ISP2 in *T. cruzi* Dm28c by generating *isp*2 null-mutants (Δ*isp*2) using CRISPR/Cas9. Δ*isp*2 was more susceptible to lysis by human serum than the parental line or transgenic lines re-expressing ISP2. Tissue culture trypomastigotes Δ*isp*2 are more infective to human muscle cells *in vitro*, which was reverted by aprotinin or by camostate mesylate, suggesting that epitheliasin (TMPRSS2) is the target of ISP2. Pre-treatment of host cells with an antagonist to the PAR2 or with an inhibitor of TLR4 selectively reversed increased host cell invasion displayed by Δ*isp*2, while it did not affect invasion by the parental or re-expressor lines. Target gene silencing of PAR2, TLR4 or TMPRSS2 in muscle cells by siRNA prevented increased host cell invasion by Δ*isp*2 in comparison to cells transfected with control siRNA primers. BALB/c infection in the footpad revealed that Δ*isp*2 induced increased tissue edema after 3h, but reduced at 24h, as compared to the parental or re-expressor lines. Additionally, the spleen of mice infected with Δ*isp*2 displayed increased population of $Ly6C^{+}Ly6G^{+}$ cells, accompanied by higher levels of KC, MCP-1, TNFα and IFNγ. We propose that that ISP2 contributes to protect *T. cruzi* from the anti-microbial effects of human serum and to prevent the engagement of inflammatory receptors in host cells, resulting in the modulation of host cell invasion, and contributing to decrease inflammation during acute infection. **Supported by:**Medical Research Council - United Kingdom (MRC-UK), CNPq, FAPERJ **Keywords:**Trypanosoma cruzi;Inibidor de Serino Proteases;CRISPR/Cas9.

HP-50 - **In silico analysis of Toxoplama gondii Calcium-dependent protein kinase 1 (TgCDPK) as potential molecular target for etiological chemotherapy**

CARVALHO, J.P.B.[1]; RAMOS, A.C.G.[2]; ADESSE, D.P.[1].
1. LABORATÓRIO DE BIOLOGIA ESTRUTURAL - IOC/FIOCRUZ, RJ - BRA; 2. LABORATÓRIO DE GENÔMICA FUNCIONAL E BIOINFORMÁTICA - IOC/FIOCRUZ, RJ - BRA.
E-mail: joao.bezerra.ismart@gmail.com

Toxoplasmosis is a disease with worldwide distribution, caused by Toxoplasma gondii. Despite the high prevalence, few treatments options are available for the global population, often related to side effects. Therefore, the search of new drugs is need for the context of toxoplasmosis. The aim of this work is to identify new therapeutic molecules against Calcium-Dependent Protein Kinase 1 from T. gondii (TgCDPK1) using computer-based drug design techniques. TgCDPK1 plays a key role in calcium-dependent exocytosis and its inhibition results in blockage of essential functions including parasite motility, host-cell invasion and egress. Although several studies have targeted TgCDPK1 in experimental models, no molecules have yet been approved. Initially, a local alignment algorithm, BLASTp, was employed, using the SwissProt database and the TgCDPK1 sequence from ToxoDB, to identify possible human homologous enzymes. We identified 11 human enzymes that had some degree of similarity with TgCDPK1, which were then aligned using CLUSTAL, a global alignment algorithm, that revealed several matching regions along the sequence, indicating proximity between them. However, in the gatekeeper residue present in the ATP binding site, we found Phe, Leu and Met residues in this position in the human sequences, whereas in TgCDPK1 we found Gly. The three-dimensional structures of the human and T. gondii enzymes were then aligned using PyMol. Through the alignment, we found structural similarities between the proteins, in addition to similar ATP binding sites. While human enzymes have bulky nonpolar residues in the gatekeeper position, TgCDPK1 has a small residue. These results may indicate structural differences in TgCDPK1 in relation to human counterparts, which could be used for the rational design of selective molecules. **Supported by:**Fiocruz, Programa INOVA, Faperj **Keywords:**Toxoplasma gondii;Molecular target;TgCDPK1.

HP-51 - **Functional characterization of *Leshmania mexicana* N-acetyltransferase NAT10 using the S. *cerevisia*e model**

ALVES, G.G.; LEITE, A.B.; PANESSA, G.M.; MARAN, S.R.; CUSSIOL, J.R.; MORETTI, N.. UNIFESP, SP - BRA.
E-mail: gabrielagms695@gmail.com

Leishmaniasis is a group of diseases that in humans is caused by more than 20 species of *Leishmania*. During its life cycle, the parasite transits between invertebrate and vertebrate hosts. To survive the environmental changes, the parasite promotes alterations in the regulation of gene expression, protein synthesis and metabolism. The gene expression in *Leishmania* is regulated mainly at post-transcriptional level, through mechanisms of mRNA stabilization/degradation and translation regulation. Several chemical modifications have been identified in mRNAs, such as N6-methyladenosine (m6A), 5-methylcytosine (m5C) and N4-acetylcytidine (ac4) and implicated in all steps of RNA lifetime. The ac4C is added to RNA by the N-acetyltransferase, NAT10, recently described as promoting an increase in the mRNAs stability and translation efficiency. Considering the crucial role of post-transcriptional mechanisms in the *Leishmania* gene expression regulation, in this work we plan to characterize functionally the L. *mexicana* NAT10 using Saccharomyces cerevisiae as study model. L. mexicana has a NAT10 homologue with all essential domains for acetyltransferase activity and RNA interaction. Thus, to investigate the parasite protein function we obtained S. cerevisiae NAT10 knockout strains using homologous recombination, replacing the NAT10 gene with the URA3 selection marker. Also, we obtained a NAT10 mutated strain that affects yeast growth at higher temperatures. Both strains will be complemented with L. *mexicana* NAT10, native and mutated at position G647 to A647. This mutation inactivates the acetyltransferase activity of NAT10. In parallel, we are generating constructs to heterologous protein expression of L. *mexicana* NAT10 to be used in further *in vitro* activity assays. The results from this work will complete ongoing studies in the parasite and will contribute to characterize the role of ac4C in *Leishmania* biology, increasing our understanding in how it regulates gene expression. **Supported by:**FAPESP - 2021/13714-8 **Keywords:**Leishmania;Acetilation;NAT10.

HP-52 - **Invasion of non-phagocytic cells by Leishmania amazonensis amastigotes: a comprehensive study of the cellular mechanisms involved in cell entry**

DE OLIVEIRA, T.Q.; MOREIRA, A.L.D.S.; BARROS, L.V.R.; HORTA, M.D.F.M.; GOMES, T.D.C..
UFMG, MG - BRA.
E-mail: thamires.olivv@gmail.com

Intracellular parasites are characterized by the need to invade and live within host cells. For Leishmania spp. it is commonly assumed that the parasite must be phagocytosed by phagocytic cells, notably macrophages. However, several studies have described the presence of Leishmania spp. amastigotes within non-phagocytic cells. Since cell entry is a key process in infections by intracellular parasites we decided to investigate the mechanisms that allow Leishmania amazonensis amastigotes to invade cells lacking the ability to perform classical phagocytosis. Here we used different cell biology techniques to study the cell invasion process of non-phagocytic cells by L. amazonensis amastigotes in mouse embryonic fibroblasts (MEF). We performed time-course infection assays, labelled infected cells and parasites to visualize molecules, organelles and structures possibly involved in cell invasion and quantified infections. Our results show that living, but not PFA-fixed amastigotes, are rapidly internalized in MEFs, where they survive and persist in typical vacuoles rich in lysosomal markers. By labeling host cells with F-actin probes and using F-actin inhibitors we demonstrated that invasion is dependent on host cell cytoskeleton. Confocal microscopies of early moments of infection indicate that the parasite is able to induce an extremely localized re-arrangement of host cell cytoskeleton to induce invasion. Since we had previously demonstrated that promastigotes use host cell lysosomes and not F-actin to invade MEFs we now propose that cell invasion of non-phagocytic cells by L. amazonensis is mechanistically different for each infective form of the parasite. The presence of Leishmania spp. amastigotes within non-phagocytic cells is an overlooked aspect of their biology. Studies on these new infection routes and on the role of non-phagocytic cells in the course of infection may provide important information about the biology of these parasites and the diseases they cause.
Supported by:CAPES **Keywords:**Leishmania amazonensis ;Amastigotes ;Cell invasion .

HP-53 - **A potential beneficial influence of Selenium treatment on cardiac function in Chagas heart disease: Results from the STCC randomized Trial**

FERREIRA, R.R.; HOLANDA, M.T.; BONECINI-DE-ALMEIDA, M.G.; MEDIANO, M.F.; HASSLOCHER-MORENO, A.M.; SARAIVA, R.M.; ARAUJO-JORGE, T.C..
FIOCRUZ, - RJ - BRA.
E-mail: robertoferreira.ioc@gmail.com

For over 60 years, Selenium (Se) is known as an essential microelement for many biological functions, including cardiovascular homeostasis. Experimental and clinical data indicate that Se may be used as a complementary therapy to prevent and improve heart failure. We have previously shown that Se levels in chronic chagasic cardiomyopathy (CCC) decrease in severe cases. We performed the first randomized, placebo-controlled, double-blinded, clinical trial designed to estimate the efficacy and safety of Se treatment in CCC. 66 patients with CCC stages B1 (n = 54) or B2 (n = 12) were randomly assigned to receive 100 mcg/day sodium selenite (n = 32) or placebo (n = 34) for one year. LVEF changes over time and adverse effects were investigated. Trial registration number: NCT00875173. No significant differences between the two groups were observed for the primary outcome. In a subgroup analysis, statistically significant longitudinal changes were observed for mean LVEF in the stage B2 subgroup (b= +10.1; p = 0.02 for Se [n = 4] vs Pla [n = 8]). Se treatment was safe for CCC patients, and the few adverse effects observed were similarly distributed across the two groups. We showed a potential beneficial effect of Se treatment in a subset of patients with CCC with ventricular dysfunction (LVEF <45%). Despite trial limitations, this is a novel therapeutic option for mild CCC. Additionally, Se may decrease the speed of worsening cardiac function and may even improve cardiac function in patients with CCC stage B2. Se treatment did not improve cardiac function (evaluated from LVEF) in CCC. However, in the subgroup of patients at B2 stage, a potential beneficial influence of Se was observed. In conclusion, the present study elucidated that Se treatment was safe and showed a potentially beneficial effect. This new pharmaceutical/nutritional approach deserves further studies to clarify its potential use as adjuvant therapy in CCC. **Supported by:**Brazilian Ministry of Health, Fiocruz, CNPq, FAPERJ. **Keywords:**Selenium ;Chagas disease;Treatment.

HP-54 - **Infection of muscle, epithelial and connective cells by *Leishmania amazonensis*: assessing parasite viability and the potential role of non-phagocytic cells as parasite reservoir**

MOREIRA, A.L.D.S.; DE OLIVEIRA, T.Q.; BARROS, L.V.R.; REIS, L.D.D.S.; MARTINS-DUARTE, É.D.S.; HORTA, M.D.F.M.; GOMES, T.D.C..
UFMG, - MG - BRA.
E-mail: anna.luiza.sm3@gmail.com

Intracellular parasites are characterized by the need to invade and exert at least part of their life cycles inside host cells. For *Leishmania* spp. it is assumed that, upon inoculation by the sandfly vector, the parasite is phagocytosed and lives inside professional phagocytic cells, notably the macrophages. However, several works have showed the presence of these parasites also in non-phagocytic cells. Muscle cells are among the non-phagocytic cell types already described as harboring intracellular amastigotes. Similarly, several epithelial and connective cells have also been found infected *in vivo*. 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. Therefore, our objective of is to study the infection of muscle, epithelial and connective cells by *Leishmania amazonensis*, to assess the viability of the parasites inside these cell types as long as their possible roles asa reservoir, ultimately leading to macrophages infection. To this end, we used light, conventional and confocal microscopies, immunobiochemical methods and flow cytometry to assess *L. amazonensis* infectivity in muscle fibers, epithelial cells and fibroblasts. Here we show that both amastigotes and promastigotes are able to invade and persist within these cells as typical intracellular amastigotes. When macrophage-like cells (RAW cells) were co-cultured with infected fibroblasts, infection rapidly spread to the macrophages, demonstrating that the parasites could be transferred from one cell to the other. Our results suggest that non-phagocytic cells may serve as a parasite reservoir that may eventually lead to macrophage infection. Future experiments will be focused on *in vivo* infection experiments using infected non-phagocytic cells as parasite donators to validate this hypothesis. **Supported by:**CNPq **Keywords:**Leishmania spp;;Cell Invasion;Non-phagocytic Cells.

HP-55 - **Transforming growth factor beta neutralization reduces Trypanosoma cruzi infection and improves the cardiac performance**

FERREIRA, R.R.[1]; DE SOUZA, E.M.[1]; PEREIRA, G.V.[1]; MEUSER-BATISTA, M.[1]; DEGRAVE, W.[1]; BAILLY, S.[2]; LANNES-VIEIRA, J.[1]; ARAUJO-JORGE, T.C.[1]; WAGHABI, M.C.[1].
1. FIOCRUZ, RJ - BRA; 2. UNIVERSITÉ GRENOBLE-ALPES, FRA.
E-mail: robertoferreira.ioc@gmail.com

Chronic Chagasic cardiomyopathy (CCC) is the most prominent clinical form of Chagas disease, culminating in heart failure and high rates of sudden death. During CCC, the parasite remains inside the cardiac cells, leading tissue damage, involving extensive inflammatory response and irregular fibrosis. Some molecules act in the fibrosis development, but one in particular plays a key role in the fibrogenic process inducing extracellular matrix synthesis: the transforming growth factor-β (TGF-β). TGF-β is also involved in the development of CCC with increased serum levels of this cytokine and activation of its signaling pathway in the cardiac tissue, resulting in increased expression of extracellular matrix proteins, which characterizes the fibrosis. The aim of this study was to investigate the effect of 1D11, a neutralizing antibody to all three isoforms of TGF-beta, on T. cruzi infection: in vitro and in vivo. To this end, cardiomyocytes were seeded for 24h, incubated with trypomastigotes and treated with 1D11. Murine models of acute and chronic Chagas disease were also treated with 1D11 with different schemes. In the present study, we show that the addition of 1D11 greatly reduces cardiomyocyte invasion by T. cruzi, in vitro. Further, the treatment significantly reduces the number of parasites per infected cell. In murine experimental models, the T. cruzi infection altered the cardiac electrical conduction: decreasing the heart rate, increasing the PR interval and the P wave duration. The treatment with 1D11 reversed this process, improving the cardiac performance and reducing the fibrosis of the cardiac tissue. Taken together, these data further confirm the major role of the TGF-β signaling pathway in both T. cruzi-infection, in vitro and in vivo. The therapeutic effects of 1D11 are promising and suggest a new possibility to treat cardiac fibrosis in the Chagas heart disease by TGF-β neutralization. **Supported by:**CAPES; CNPq; FAPERJ and Fiocruz-RJ. **Keywords:**Chagas disease;TGF-beta;1D11.

HP-56 - **TLR4-PAR2 crosstalk influences macrophage infection by *Leishmania braziliensis.***

RODRIGUES, N.S.; GOUNDRY, A.L.; LIMA, A.P.C.D.A..
INSTITUTO DE BIOFÍSICA CARLOS CHAGAS FILHO, RJ - BRA.
E-mail: nsrodrigues@biof.ufrj.br

The host serine peptidase neutrophil elastase (NE) has been shown to control *Leishmania major* infection both in macrophages *in vitro* and in *in vivo* animal models. However, *L. major* possesses an inhibitor of S1A serine peptidases, known as ISP2, which inhibits the activity of NE, downregulating phagocytosis during macrophage interaction but promoting intracellular growth via the blockade of a pathway associated with Toll-like receptor 4 (TLR4). In contrast to *L. major*, we show that the internalization of *L. braziliensis* is negatively affected when NE is inhibited by the addition of exogenous NE inhibitor prior to macrophage infection or when NE is absent, as in the case of macrophages from NE-knockout mice (*ela2*$^{-/-}$). A similar profile was seen when TLR4 was inhibited with a commercial inhibitor (TAK-242). Furthermore, proteinase-activated receptor 2 (PAR-2) has previously been shown to interact with TLR4. Likewise, the uptake of parasites was decreased when macrophages were pre-incubated with a commercial antagonist of PAR-2 (AZ3451). When the TLR4 inhibitor and PAR-2 antagonist were used together the rate of internalization was the same as when either was used alone suggesting that they act in the same pathway. Interesting, macrophages derived from transgenic mice lacking another S1A serine peptidase, cathepsin G, exhibited a higher rate of uptake of *L. braziliensis* and the infection was sustained until 72 h. Together, our data indicates that Leishmania braziliensis engages a neutrophil elastase-dependent TLR4-PAR2 crosstalk to infect macrophages. **Supported by:**CNPq, Faperj, CAPES **Keywords:**Leishmania braziliensis;Inibidor de Serino Proteases;Infection .

HP-57 - **Replication control in *Trypanosoma cruzi***

SANTAROSSA, B.A.[1]; KELLY, J.M.[2]; CALDERANO, S.G.[1].
1. INSTITUTO BUTANTAN, SP - BRA; 2. LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE, GBR
E-mail: simone.calderano@butantan.gov.br

MCM2-7 is a heterohexamer complex with a ring shape that can encircle DNA and unwind it during replication. This complex along with other replication factors must be activated in S phase, so DNA replication can be initiated. Also, MCMs are involved in DNA replication control by being exported from nucleus or degraded when DNA replication is no longer happening inside the nucleus. During *Trypanosoma cruzi* life cycle there are lifeforms capable of replicating itself and lifeforms that does not replicate. Epimastigotes are replicating forms responsible for *T. cruzi* expansion inside the insect vector. This lifeform must control its replication to generate two daughter cells with high copy fidelity and when transforming to tripomastigotes metacyclics. So, this works aims to understand how MCMs are involved in replication control in *T. cruzi*. Five subunits of the mcm2-7 complex where tagged with neon green protein using CRISPR/Cas9 genetic editing tool. Fluorescence images showed that Mcm 2,3,4,6 and 7 are found in the nucleus of epimastigote forms and colocalized with EdU, a DNA replication marker. This nuclear pattern was observed in all cell cycle phases of epimastigotes for the five proteins tagged. For further characterization knockouts of MCM 6 and 7 were generated by CRISPR/Cas9. While a double KO of MCM7 was obtained, only single KO of MCM6 were generated. These cell lines showed impairment in cell cycle growth and the double KO of MCM7 presented a population of cell with DNA content greater then G2 population identified by flow cytometry. Single KO of MCM6 and 7 did not presented changes in DNA content. These data indicates that the genes identified as MCMs in *T. cruzi* genome are indeed involved in DNA replication, are essential for *T. cruzi* replication and are involved in genomic stability. However, until now MCMs do not seem to participate in DNA replication control once they are expressed in all cell cycle phases and are always confined to the nucleus. **Supported by:**FAPESP 202101013-5 **Keywords:**cell cycle;replication control;Trypanosoma cruzi.

HP-58 - **Evaluation of the role of ATP-citrate Lyase on macrophage polarization during *Leishmania amazonensis* infection**

GOMES, J.S.D.C.; SARAIVA, E.M..
UFRJ- RJ - BRA.
E-mail: jangomes93@gmail.com

Leishmaniases are neglected tropical and subtropical diseases caused by *Leishmania* parasites. About 20 different species of the genus *Leishmania* are known to cause the disease, consisting of a spectrum of clinical forms. *Leishmania* infects macrophages, surviving the phagolysosomal microbicidal mechanisms. It is important to understand the changes caused in macrophages during *Leishmania* infection and how these changes favor parasite survival. Classically (M1) and alternatively (M2) activated macrophages undergo metabolic reprograming, but differ in some aspects. ATP citrate lyase (ACLY) is involved in macrophage polarization towards M1 and M2 profiles. It uses citrate to produce acetyl-CoA and oxaloacetate, which are used to produce inflammatory mediators and induce histone acetylation. Recent work has shown that *Leishmania* parasites can induce metabolic reprograming in macrophages. In *L. amazonensis* infected macrophages, there is a switch in the metabolism. In *Leishmania* infected bone marrow-derived macrophages (BMDMs) OXPHOS and glucose metabolism are increased. Based on these findings, we hypothesized that macrophages undergo metabolic reprogramming when infected with *L. amazonensis*, leading to an increase in metabolite citrate. ACLY then uses citrate to produce acetyl-CoA, which leads to histone acetylation and modulation of macrophage response to the infection. Here, we are testing the ACLY enzyme's activity and describing its function during *L. amazonensis* infection in macrophages. Our preliminary results show that pharmacological inhibition of ACLY reduces parasite survival in infected macrophages. The enzyme inhibition did not affect promastigotes viability, suggesting that the effect was due to modulation of macrophage response. Moreover, ACLY inhibition enhanced ROS production in infected BMDMs. Based on these results, we consider that ACLY may contribute to macrophage polarization towards the M2 profile and may be a target for leishmaniasis treatment. **Supported by:**CNPq, CAPES, FAPERJ **Keywords:**L; amazonensis;metabolic reprograming;ATP citrate lyase.

HP-59 - ***Prediction, production and evaluation of chimeric proteins as an antigen for serological diagnosis of Chagas' disease.***

ITURRA, J.A.D.; TEIXEIRA, S.M.R.; FIGUEIREDO, M.M.; DA SILVA, L.B.; MACHADO, L.F.M.; FERNANDES, A.P.S.M..
LABORATORY VACCINE TECHNOLOGY CENTER (CT-VACCINAS) - FEDERAL UNIVERSITY OF MINAS GERAIS, MG - BRA.
E-mail: jaraujo1904@gmail.com

Chagas' disease (ChD) is a neglected disease endemic in 21 Latin American countries. According WHO and DND*i*, 6 to 7 million people are affected by ChD, with estimated 75 million people at risk of contracting the disease and an average of 14,000 deaths each year. ChD is caused by *Trypanosoma cruzi*, but less than 10% of carriers have access to diagnosis and less than 1% receive treatment. Recombinant proteins may improve diagnosis which is an essential task among the strategies to control and prevent this disease. However, diagnosis based on a single recombinant antigen may not achieve high sensitivity, given the antigenic diversity seen inside *T. Cruzi* lineages and strains. Here, we developed chimeric antigens for to improve sensitivity for serodiagnosis of ChD. Bioinformatics analyzes were performed for *T. cruzi* proteins previously described and already tested as single antigens, to identify linear epitope sequences of B cells. The nucleotide sequences obtained were joined into synthetic genes, inserted into pET-24a(+) vector with a histidine tag to transform *E. coli* bacterial strains. Using a different combination of epitopes, three chimeric proteins were proposed: QTc1 (28,27 kDa); QTc2 (26,73 kDa) e QTc3 (30,8 kDa), with Isoelectric Point 6,53; 9,38 e 5,18, respectively. Protein production conditions were evaluated, followed by purification by affinity chromatography. QTc1 and 2 were induced for 3 hours at 37°C and QTc3, for 18 hours at 20°C. All proteins proved to be soluble. A Western Blot test was performed, using sera from healthy and chagasic individuals. The results suggest that QTc1 and 2 are promising antigens. The purified proteins will be used as antigens in ELISA and TR with standard sera samples to determine parameters (sensitivity, specificity, among others). Then, selected conditions will be further explored to prepare prototype tests for validation as alternative for the diagnosis of Chagas' disease. **Keywords:**serodiagnosis;Chagas' disease;chimeric antigens.

HP-60 - **IMPACT OF TOXOPLASMA GONDII INFECTION ON SKELETAL MUSCLE MYOGENESIS IN A THREE-DIMENSIONAL CULTURE MODEL**

LOPES, C.E.; JORGE, R.D.S.; VIEIRA, P.D.C.; BARBOSA, H.S.; ADESSE, D.P..
FIOCRUZ, RJ - BRA.
E-mail: carolinaepifaniolo@gmail.com

Toxoplasma gondii is the etiological agent of toxoplasmosis and has the skeletal muscle tissue as one of the main niches where tissue cysts are formed. For this reason, the skeletal muscle cell-T. gondii interaction has been subject of many studies from our group. In vitro studies related to this infection were performed in monolayer cultures, and we described that T. gondii impairs myogenesis in mouse myoblast cultures. Three-dimensional (3D) cell culture models better mimic the cellular organization of the tissues in vivo and have been increasingly used in research for drug discovery, developmental biology and infectious diseases. In the present work, we generated 3D cultures (spheroids) of the C2C12 mouse myoblast cell line to evaluate the infectivity rates and myogenesis events after infection by T. gondii. Cells were plated in agarose-coated 96-well U-bottom plates, and after 24 hours were infected with T. gondii tachyzoites (ME49 strain) in a 1:1 ratio. After 24 hours of infection, half of the plate had its proliferation medium changed to a differentiation medium that induces the myogenic program. Five days after myogenesis induction (that corresponds to 6 days of infection) we measured the area of the spheroids and performed immunostainings for myosin heavy chain (MyHC, a marker of differentiated myocytes), desmin (marker of the muscle cell lineage) and SAG1 (for tachyzoites). After 24 hours of differentiation, no changes were observed regarding the spheroids size, but at 120 hours after differentiation, a significant increase in spheroids surface was noticed, in infected cultures kept both under proliferation and differentiation media. T. gondii infection was detected throughout the spheroids by immunostaining and was accompanied by the reduced immunoreactivity of MyHC and desmin. This new model will be of great relevance to further understanding the mechanisms involved in skeletal muscle pathology induced by T. gondii infection. **Supported by:**PIBIC, CNPq, Faperj, INOVA Fiocruz, PAPES VII Fiocruz **Keywords:**T;gondii;Muscle cells;spheroids.

HP-61 - **Lipophosphoglycan-3 protein from *Leishmania infantum chagasi* plus saponin adjuvant: A new promising vaccine against visceral leishmaniasis**

EMERICK, S.D.O.[1]; DE CARVALHO, T.V.[2]; MIRANDA, B.M.[1]; DA SILVA, A.C.[1]; MARTINS, T.V.F.[1]; RAIMUNDO, V.D.[1]; DE OLIVEIRA, L.L.[1]; MARQUES-DA-SILVA, E.D.A.[1].
1. UNIVERSIDADE FEDERAL DE VIÇOSA, MG - BRA; 2. UFOP, MG - BRA.
E-mail: vagner.raimundo@ufv.br

Remoto Poster Session

Given the lack of a vaccine against leishmaniasis, this work contributes to the development of a vaccine against visceral leishmaniasis. In this study, BALB/c mice were immunized with *L. chagasi* LPG3 or rLPG3 proteins alone or associated with incomplete Freund's adjuvant (IFA) or saponin (SAP) and challenged with *L. chagasi*. The parasite load in the spleen and liver and the immune response of the groups were compared. Among the formulations tested, LPG3 + SAP reduced about 98% of the parasite load in the spleen and liver, increased the production of cytokines IL-2, IFN-γ, IL-6, IL-17 and IL-10 by stimulated spleen cells by LcAg and reduces the IgG1/IgG2a ratio. Vaccine with rLPG3 induced Th1/Th17 response, produced IL-10 and protected the spleen of mice infected with *L. chagasi*. A reduction in parasitism was observed in the spleen of mice vaccinated with rLPG3 and rLPG3+SAP. LPG3 has already been investigated to stimulate an immune response, and it has been found to be able to do so if it is combined with an adjuvant. Choosing the right adjuvant is crucial in producing a vaccine to induce an effective response. In our results, SAP was more effective than IFA in providing protective immunity through the use of native and recombinant LPG3. In fact, SAP has been used in leishmaniasis vaccine formulations to induce a Th1 immune response. The rLPG3 + SAP vaccine suggests safety, as it preserves liver architecture and function. It is also noteworthy that the production of rLPG3 is easier and cheaper compared to current processes for obtaining the native protein. Work approved by the Ethics Committee for Animal Use (CEUA/UFV – process 16/2016), financed by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG – Grant No. 9553 – FAPEMIG CBB – APQ-00668-13), Coordination Improvement of Higher Education Personnel (CAPES) and National Council for Scientific and Technological Development (CNPq) MCTI/CNPq/MEC/Capes – Transversal Action 06/2011 – Casadinho/Procad. **Keywords:**LPG3;Saponin;Visceral leishmaniasis.

HP-62 - **Lipophosphoglycan-3 recombinant protein vaccine controls hepatic parasitism and prevents tissue damage in mice infected by *Leishmania infantum chagasi***

BASTOS, D.S.S.[1]; MIRANDA, B.M.[1]; MARTINS, T.V.F.[1]; ERVILHA, L.O.G.[1]; SOUZA, A.C.F.[2]; EMERICK, S.D.O.[1]; DA SILVA, A.C.[1]; RAIMUNDO, V.D.[1]; NOVAES, R.D.[3]; NEVES, M.M.[1]; SANTOS, E.C.[1]; DE OLIVEIRA, L.L.[1]; MARQUES-DA-SILVA, E.D.A.[1].
1. UFV, MG - BRA; 2. UFRRJ, RJ - BRA; 3. UNIFAL, - MG - BRA
E-mail: vagner.raimundo@ufv.br

Remoto Poster Session

In this work, immunization with recombinant LPG3 protein was tested on the parasitism and on the liver of infected BALB/c mice, because this is one of the target organs for the parasite. The vaccination protocol consisted of three doses for five groups: uninfected and treated with PBS (NI), group challenged and treated with PBS (NV), saponin (SAP), group immunized with rLPG3 and group immunized with rLPG3 + SAP. Mice were challenged with 1×10^7 *L. infantum chagasi* promastigotes and subsequently sacrificed. rLPG3 and rLPG3 + SAP reduced parasitism, 93.82% and 99.33% respectively, compared to NV. rLPG3, with or without SAP, reduced the ratio of IgG1/IgG2a antibodies compared to the NV group. Immunization with rLPG3 + SAP was more effective, with levels of liver damage markers similar to the NI group, increased antioxidant enzyme activity and preserved liver architecture. The lower ratio of IgG1/IgG2a antibodies suggests that immunization with rLPG3 + SAP promoted immunity with a Th1 profile, reducing the parasite load. This reduction is also due to the activation of macrophages by IFN-γ, which activates infected cells and induces the production of nitric oxide and free radicals that kill the parasites. Immunization with rLPG3 + SAP can reduce the proinflammatory response that could affect organ structure. Additional studies are needed to clarify the immune mechanism triggered by immunization with rLPG3 plus SAP. However, the results of this work highlight the importance of vaccination, not only as an antiparasitic mechanism, but also as a factor that acts to maintain the integrity of the infected organ. Work approved by the Ethics Committee for the Use of Animals (approved by the CEUA/UFV – Research Project – process: 16/2016). Support: Fundação de Amparo à Pesquisa do Estado de Minas Gerais [grant APQ-01211-17 to EAM] and Coordination for the Improvement of Higher Education Personnel [doctoral fellowship for DSSB]. **Keywords:**LPG3;Visceral leishmaniasis;Liver morphology.

HP-63 - **Histological analysis of the seminiferous tubules and intertubular area of mice infected by the Colombian strain of *Trypanosoma cruzi.***

DOS SANTOS, B.L.P.[1]; MENEZES, T.P.[2]; SILVA, V.L.T.E.[1]; DIAS, F.C.R.[3]; RIBEIRO, L.[1]; PINTO, K.M.D.C.[2]; DA SILVA, A.T.P.[2].
1. UFMG, MG - BRA; 2. UFOP, MG - BRA; 3. UFTM, MG - BRA.
E-mail: breno.psantos@yahoo.com.br

Remoto Poster Session

Chagas' disease is a tropical neglected illness caused by *Trypanosoma cruzi* and remains one of the most significant causes of morbidity and mortality in South and Central Americas. The disease is caused by a moderate to intense and persistent inflammatory response characterized by local upregulated expression and production of inflammatory mediators (such as cytokines and chemokines) that favors the activation and recruitment of distinct cells of the immune system into different tissues to eliminate the parasites. Heart, muscular and tissues of the gastric system are mostly common to be studied as a regular tissue tropism of the *T. cruzi*, but the protozoan can infect and cause an inflammatory response in several systems on its host, such as the reproductive system. This research focused on the testicular investigation in mice infected by 10^3 trypomastigote forms of Colombian strain of *T. cruzi*. Its was evaluated histological parameters of the seminiferous tubules and the intertubular area of 14 animals grouped as: control (non-infected) and *T. cruzi*. The luminal diameter and area increased in the presence of the protozoan resulting in a different seminiferous tubules/epithelial relation between the control and the *T. cruzi* group. The percentage of the seminiferous tubules in the group infected by the protozoan are also higher than the control group, such as the percentage and volume of the tunica propria. In the intertubular area, the percentage of the intertubule was higher in the control group, such as the percentage and volume of Leydig cells, connective tissue, and macrophages. The knowledge that testicular structures are changed by *T. cruzi* brings to the light to an important issue about a neglected route infection of the Chagas' disease: the sexual transmission. **Supported by:**FAPEMIG **Keywords:**Trypanosoma cruzi; Spermatozoids; Seminiferous tubule.

HP-64 - ***Theracurmin* regulates parasite and inflammatory mediators in mice infected by Colombian strain of *Trypanosoma cruzi*.**

DOS SANTOS, B.L.P.[1]; SILVA, V.L.T.E.[1]; MENEZES, T.P.[2]; PINTO, K.M.D.C.[2]; DA SILVA, A.T.P.[2].
1. UFMG, MG - BRA; 2. UFOP, MG - BRA.
E-mail: breno.psantos@yahoo.com.br

Remoto Poster Session

Theracurmin is a curcumin's derived formulation of nanoparticles. Its anti-inflammatory properties make this bioactive compound a mitigating factor in pathological cases after an overwhelming inflammatory response. The *Trypanosoma cruzi*, the etiologic agent of Chagas' disease, triggers an acute inflammatory response characterized by the expression/release of inflammatory mediators that favors the activation and the recruitment of distinct immune cells into different tissues to eliminate the parasite. The present research evaluated the effectiveness of the *Theracurmin* (CurcuminRich®, Natural Factors, Canada) treatment over the inflammatory and parasitological response of 32 male mice, Swiss lineage, infected by 10^3 trypomastigote forms of the Colombian strain of *T. cruzi*. These animals were grouped as: uninfected, *T. cruzi*, *T. cruzi* + *Theracurmin*, and *Theracurmin*. The mice were treated with 30 mg/Kg of *Theracurmin* (by gavage) during the period of 30 days and parasitemia was evaluated daily. At the 30th day post infection animals were euthanized, and its testicles, heart, gastrocnemius fragments and 1mL of blood collected to morphological and immunological assays. The animals infected and treated with *Theracurmin* presented a reduction in the parasitemia and in the levels of IL-15 (testicles, heart, and gastrocnemius) CCL2 (heart) and IL-6 (testicles). Therefore, it is concluded that the *Theracurmin* acts on controlling blood parasitic and on the tissue production of inflammatory mediators (IL-15, CCL2, and IL-6) in distinct tissues. **Supported by:**Fapemig **Keywords:**Trypanosoma cruzi;Theracurmin;Inflammatory response.

HP-65 - **Annexin A1 regulates inflammatory response and development of toxoplasmosis**

RABELO, R.A.N.; BARBOSA, C.L.N.; PORTO, S.; PEREIRA, R.D.D.; QUEIROZ JUNIOR, C.M.; FAGUNDES, C.T.; MACHADO, F.S..
UFMG, MG - BRA.
E-mail: rayane.rabelo10@gmail.com

Remoto Poster Session

Toxoplasma gondii(*Tg*) is arguably the most successful parasite because, in part, of its ability to infect and persist in most warm-blooded animals. A unique characteristic of *Tg* is its ability to persist in the SNC of a variety of hosts, including humans and rodents. The treatment is ineffective, so studies with new therapeutic approaches are needed. The immune system is fundamental for the control and pathophysiology of toxoplasmosis. Annexin A1, a pro-resolving and anti-inflammatory protein is induced by glucocorticoid hormones that mediate several actions of this class of drugs. Macrophages(MOs) and glial cells are crucial in controlling this infection. Herein, the aim was to evaluate the role of ANXA1 protein during an *Tg* experimental infection, *in vitro* and *in vivo*. Peritoneal MOs and glial cells from Balb/c (WT) and ANXA1 knockout (KO) mice were infected with tachyzoites forms of *Tg* RH strain (ratio 1:1 - cell:parasite). WT and KO mice aged 8 to 9 weeks were infected or not with 20 cysts intraperitoneally. The body weight and survival of the mice was monitored, every 5 days after infection (dpi) and daily, respectively. On the 25 dpi, the mice were euthanized and the brain removed for cysts counting. Brain histological analyzes of WT and KO animals were performed at 25 dpi. *In vitro*, MOs and glial cells from KO mice were more vulnerable to parasite replication compared to WT cells.*In vivo*, the deficiency of ANXA1 resulted in increased susceptibility to *Tg* infection when compared to WT. Of note, the vast majority of infected KO mice death occurred at 10 dpi. The weight loss was similar between infected groups. Furthermore, KO mice presented a higher number of brain cysts and more severe lesion in the cortex compared to WT mice. Suggesting that the absence of ANXA1 in the brain cells facilitate the parasite infectivity, or those cells have less ability to control the parasite replication. Collectively, our data suggest the ANXA1 is a regulator of *Tg* infection. **Supported by:**CNPq, Capes, Fapemig **Keywords:**Toxoplasma gondii;annexin A1;macrophages.

HP-66 - **Improved efficacy of breathing and neuroprotection effects of Amido-Coumarin co-treatment during severe experimental malaria**

LEITE, P.G.[1]; SOARES, A.T.C.[1]; PORTO, S.[1]; ROCHA, L.K.[1]; OLIVEIRA, N.F.D.M.[1]; SANTOS, L.L.D.O.[1]; OLIVEIRA, F.B.R.[1]; BARBOSA, C.L.N.[1]; RABELO, R.A.N.[1]; SANTANA, L.F.D.[1]; GOMES, F.T.B.[1]; BASTOS, M.C.D.S.[1]; PEREIRA, R.D.D.[1]; TEIXEIRA, M.M.[1]; RUSSO, R.C.[1]; MATOS, M.J.[2]; MACHADO, F.S.[1].
1. UFMG, MG - BRA; 2. UNIVERSIDADE DE SANTIAGO DE COMPOSTELA, ESP
mail: paulogaioleite@gmail.com

Remoto Poster Session

*Plasmodium berghei*ANKA(PbA)infection in mice closely recapitulates many aspects of severe malaria in humans including cerebral malaria and acute respiratory distress syndrome.Coumarins are a class of secondary metabolites present in plants and exhibit several biochemical effects.We aimed investigate the potential therapeutic of a coumarin compound in treating of severe experimental malaria(SEM).C57Bl/6mice were inoculated with 10^5red cells parasitized with PbA, and3days after infection(dpi) orally treated daily with coumarin compound MJM alone and/or combined with chloroquine(CQ) once per day.Analyzed:parasitemia,body weight,survival,clinical score,memory, and immune cell in: spleen, brain and bronchoalveolar lavage(BAL) by flow cytometry;lung conditions was evaluated by spirometry.Our results showed that the treatment with MJM alone reduced the parasitemia at 6 and7dpi, and improved the clinical score from 8to12dpi when compared with infected untreated mice.The animals treated with MJM+CQ showed a reduction in parasitemia in all analyzed kinetics(3-47dpi).Treatments with MJM and MJM+CQ increased the survival of infected mice(28dpi and 47dpi).Treatment with MJM resulted in the protection of cognitive ability at 5dpi, and the combined treatment of MJM+CQ preserved cognition at 5dpi and 47dpi.Treatment with MJM+CQ resulted:in the brain,reduction in the numbers(nº) of macrophages,dendritic cells,CD4+and CD8+IFNγ+,CD4+IL17+and IL10+,and in the spleen, reduction in the nº of neutrophils,CD4+IFNγ+orIL17+,and increased nº of CD8+IFNγ+.Spirometry results showed that,compared with infected untreated mice, or mice treated with MJM or CQ alone, the animals treated with MJM+CQ improved their lung capacities/functions.Treatment with MJM+CQ significantly increased the nº of alveolar macrophages IL10+, and CD4+IL17+ in the BAL.Collectively, our results suggest that the compound MJM,has potentially beneficial effects on neuroprotection and respiratory capacity during SEM. **Supported by:**CNPq (474971/2013-9) **Keywords:**Cerebral malaria;neuroprotection;MA-ARDS.

HP-67 - **Kinetic characterization of enzymes Enoyl-CoA hydratase, Isovaleryl-CoA dehydrogenase and GPR domain of Pyrroline-5-carboxylate synthase in *Trypanosoma cruzi* using a machine learning algorithm applied to time-course curves**

ACHJIAN, R.W.[1]; GAMEZ, K.O.[2]; CASTAÑEDA, S.D.C.M.[1]; MARCHESE, L.[1]; MICHELS, P.[3]; SILBER, A.M.[1].
1. USP, SP - BRA; 2. TU DELFT, HOLANDA; 3. UNIVERSITY OF EDINBURGH, SCO
E-mail: renan.achjian@usp.br

Remoto Poster Session

Amino acid metabolism pathways play a significant role in the biology of *T. cruzi* besides protein synthesis. As an example, proline has been shown to be involved in the energetic sustenance of the parasite, by transferring electrons to the Electron Transport Chain, and its steady state concentration levels vary widely between parasite stages. In order to better understand these pathways, our group sought to biochemically characterize enzymes for which there was little or no data available in the literature. In particular, this work focuses on TcECH and TcIVDH, from the branched chain amino acids degradation pathway, and on the GPR domain of the bifunctional TcP5CS, from the proline biosynthesis pathway. After cloning their correspondent genes, expressing and purifying the proteins, and measuring their biochemical activity under varying concentrations of substrates, we sought to obtain the kinetic parameters of the enzymes. To achieve this, we developed an algorithm that fully utilizes the whole time course curves, instead of just approximating the slope of the initial phase. In doing so, we not only take advantage of all available data for increased accuracy, but also reduced the experimentalist bias. Moreover, our algorithm is able to correct for slight protein quantification and pipetting errors. The basic strategy is to use either a gradient descent or a genetic algorithm to infer the parameters that minimize the quadratic error between simulated and experimental time courses, assuming that the Uni-Uni or Bi-Bi Reversible Hill Equation applies (though the user is able to provide any other equation with minimal adjustments). Failures of the algorithm are also informative, as they reveal where and why more data is needed, and can highlight situations where results appear to be consistent, but fail to be reproducible, requiring improvements on sample preparation and handling protocols. **Supported by:**FAPESP: 2020/04482-3 **Keywords:**Kinetic characterization;Amino acid metabolism;Time-course curves.

HP-68 - ***Trypanosoma cruzi* and betacoronavirus MHV3 co-infection: the role of SOCS2 in the systemic manifestation.**

BARBOSA, C.L.N.; PEREIRA, R.D.D.; RABELO, R.A.N.; QUEIROZ JUNIOR, C.M.; OLIVEIRA, N.F.D.M.; OLIVEIRA, F.B.R.; SANTOS, L.L.D.O.; SANTANA, L.F.D.; BASTOS, M.C.D.S.; SOARES, A.T.C.; MACHADO, F.S..
UFMG - UFMG, MG - BRA.
E-mail: cesarbarbosa_91@yahoo.com.br

Remoto Poster Session

Patient with Chagas disease (CD), caused by *Trypanosoma cruzi* (TC) infection, has no symptoms or present gastrointestinal/myocardial dysfunction. However, coinfections can lead to CD reactivation and influence the overall prognoses. COVID-19, generated by Sars-cov2 can lead respiratory and systemic pathophysiological manifestations/death. Mice inoculated with betacoronavirus murine hepatitis coronavirus 3 (MHV3) develops severe disease and it is an animal model for studies regarding betacoronavirus respiratory infection and related diseases. The SOCS2 protein is regulator of the innate/adaptive response to different infections/homeostasis. Herein, we evaluate the role of SOCS2 at the chronic phase of TC infection, MHV3, and TC/MHV3 coinfection in mice. C57BL/6 (WT) and SOCS2 knockout (KO) animals were infected with TC Y strain and at the chronic phase (100 days after infection; dpi) we performed the MHV3 co-infection. A reduction of lymphocytes was observed in the blood of WT and SOCS2 KO, and the deficiency of SOCS2 resulted in decreased numbers of granulocytes when compared with WT at 100 dpi. During MHV3 infection, was observed a lymphopenia being more significative in SOCS2 KO at 3dpi, and the deficiency of SOCS2 also dramatically increased eosinophils and monocytes numbers. The co-infection (TC+MHV3) resulted in restauration of circulating lymphocytes numbers that was dependent of SOCS2. The histology analyses demonstrated that absence of SOCS2 during TC and MHV3 infection increased the development of inflammation and lesion in the liver and gut. Interesting, in the co-infection, SOCS2 KO mice was more resistant to inflammatory development in the lung and liver, but more susceptible in the gut, that was associate with an increased Enterobacteriaceae, when compared to WT. Altogether, the results demonstrated the critical role of SOCS2 regulating the inflammatory response and tissue protection during TC, MHV3 and TC+MHV3 co-infection process. **Supported by:**CAPES, CNPQ e FAPEMIG **Keywords:**SOCS2;Inflammatory;Co-infection.

HP-69 - **Effect of zileuton treatment *in vivo* and *in vitro* during *Trypanosoma cruzi* infection**

OLIVEIRA, F.B.R.; PEREIRA, R.D.D.; RABELO, R.A.N.; ARAÚJO, R.R.S.; MACHADO, F.S..
UFMG, MG - BRA.
E-mail: fernandobento421@gmail.com

Remoto Poster Session

Chagas disease (CD) is a neglected disease caused by the protozoan *Trypanosoma cruzi*. The main complication of CD includes cardiac, digestive and neurological dysfunction. Among the challenges for the treatment of disease, there is the inefficiency of drugs available for the chronic phase. There are studies demonstrating that, during the *T. cruzi* infection, the host immune response could be regulated by the action of eicosanoids, including Lipoxins (LXA) that induces the expression of the protein Suppressor of Cytokine Signaling 2, an important regulator of immune response. Moreover, pathogens can evade immune response producing/expressing mediators that mirror the mammalian and plant molecules, including enzyme-like lypoxigenase, for instance the lypoxigenase expressed by *Toxoplasma gondii*. Zileuton (Zi) is a selective inhibitor of 5-lipoxygenase (5-LO) enzyme which is involved in the intracellular pathways that induce leukotrienes and LXA production. Herein, we investigate the effects of Zi, *in vitro* on trypomastigote and epimastigote forms of the parasite, and during experimental *T. cruzi* infection *in vivo.* Female C57BL/6, SV129 and 5-LO knockouts (KO) mice, 6 to 8 weeks old, were infected intraperitoneally with 1000 forms of trypomastigotes Y strain and treated or not with Zi (30mg/kg) or benznidazole (Bz; 10mg/kg). The treatment started 8 hours after infection, and was conducted each 12 hours for 10 days. *In vitro***,** incubation of trypomastigotes and epimastigotes with Zi (at 100, 33, 10, 3 and 1µM), but not with dimethyl sulfoxide (DMSO; vehicle control), for 72h, reduced the number of parasites. *In vivo***,** treatment with Zi and Bz reduced significantly the parasitemia when compared to untreated animals. The control of parasitemia during Zi treatment was partially dependent of 5-LO. Collectively, these preliminary results suggest that zileuton has a potential protective effect against T. *cruzi* infection acting in both host and parasite cells. **Supported by:**CNPq, FAPEMIG, CAPES. **Keywords:**Trypanosoma cruzi;Zileuton;5-lipoxygenase.

HP-70 - **Effects of formyl peptide receptor 2 deficiency in the modulation of immune response and development of pathogeneses during Plasmodium berghei ANKA**

SANTOS, L.L.D.O.[1]; LEITE, P.G.[1]; SOARES, A.T.C.[1]; PORTO, S.[1]; ROCHA, L.K.[1]; OLIVEIRA, N.F.D.M.[1]; OLIVEIRA, F.B.R.[1]; BARBOSA, C.L.N.[1]; RABELO, R.A.N.[1]; SANTANA, L.F.D.[2]; GOMES, F.T.B.[1]; BASTOS, M.C.D.S.[1]; PEREIRA, R.D.D.[1]; TEIXEIRA, M.M.[1]; RUSSO, R.C.[1]; MACHADO, F.S.[1].
1. UFMG, MG - BRA; 2. UFMG, CONTGEM - MG - BRA.
E-mail: lauralis.oli@gmail.com

Remoto Poster Session

Severe malaria caused by Plasmodium falciparum in humans can results in high morbidity and mortality. The Plasmodium berghei ANKA (PbA) infection in mice closely recapitulates many aspects of human severe malaria, including respiratory distress and cerebral malaria. Formyl peptide receptor 2 (FPR2) is involved in the organism response to infections and plays an important role in the anti-inflammatory/resolution pathway presenting high affinity for lipoxin A4 (LXA4). FPR2 has been associated with the pathogenesis of sterile and infectious inflammatory diseases. However, the role of FPR2 in malaria is unclear. C57BL/6 (WT) and FPR2 knockout (KO) C57Bl/6 mice were inoculated with 105 red cells parasitized with PbA. Despite similar parasitemia found between WT and FPR2 KO mice, deficiency of FPR2: increased the numbers of macrophages producing IL-10; but decreased the numbers of CD4+ T cell producing IL-10; and decreased the numbers of CD8+ T cells producing IFN-gamma in the bronchoalveolar lavage (BAL) at 5 days after infection when compared with WT counterparts. In the brain, absence of FPR2 increased the frequency of PbA-infected erythrocytes detected/adhered in the vein when compared with WT. Moreover, at 5 dpi, a dramatically decreased of the number of CD4+ and CD8+ T cells producing IL-17 and CD8+ T cells producing IFN-gamma was observed in PbA-infected FPR2 KO mice when compared with WT counterparts. Collectively, our results suggested that during the PbA infection the FPR2 receptor is important controlling brain parasitism and orchestrating the immune response development. **Supported by:**Financial support: CNPq CAPES, Fapemig **Keywords:**MALARIA;FPR2;LIPOXIN A4.

PV-03 - **Endosymbiosis in trypanosomatids: the presence of the symbiotic bacteria suppresses the overflow metabolism in *Angomonas deanei***

DOS SANTOS JUNIOR, A.B.[1]; MACHADO, A.C.L.[2]; DE SOUZA, W.[1]; MOTTA, M.C.M.[1].
1. IBCCF - UFRJ, RJ - BRA; 2. CENABIO - UFRJ, RJ - BRA.
E-mail: azuil@biof.ufrj.br

The coevolution of *Angomonas deanei* with a symbiotic bacterium represents an important model for studying the origin of organelles and parasitism. There is an intense metabolic exchange between both partners and the symbiont completes essential biosynthetic pathways of the host protozoan. In this work, we investigated the influence of the symbiont on the intermediary and energetic metabolism of *A. deanei* through ultrastructural and biochemical analyses. The wild-type (AdWt) and apossymbiotic (AdApo) strains were compared under different nutritional conditions. Results showed that AdWt incorporated and consumed less glucose than AdApo. Nuclear Magnetic Resonance (NMR) revealed that ethanol is twice more excreted by AdApo than by AdWt. Proteomic analyzes indicate that fermentation pathway enzymes were upregulated in AdApo, while AdWt upraised Krebs cycle and oxidative phosphorylation enzymes. A 24% reduction in ATP production was observed in AdWt cultured in SDM80 with glucose or in Warren medium after KCN inhibition, but this result was not repeated when cells were cultured with proline or submitted to fasting. AdApo showed no significant variation in ATP levels after growth under different conditions or inhibition by KCN. O_2 consumption by AdWt cultured with glucose was higher than that obtained with proline or submitted to fasting, while in AdApo no differences were observed in these culture conditions. Ultrastructural analyzes suggest that in the presence of proline or in fasting state, cells showed enlarged mitochondrial cristae, whereas in SDM80 with glucose mitochondrial swelling and approximation between glycosomes and mitochondrion were observed. Data indicate that the symbiont promotes the recovery of intermediate metabolites, reducing the overflow and optimizing the metabolism of *A. deanei*. **Supported by:**CAPES / 88887.604982/2021-00 **Keywords:** endosymbiosis;intermediate metabolism;energy metabolism.

PV-04 - **Characterization of Crithidia-like parasites isolated from human visceral leishmaniasis cases in Brazil.**

ROGERIO, L.A.[1]; TAKAMIYA, N.T.[1]; TAKAHASHI, T.Y.[1]; TORRES, C.[1]; COSER, E.M.[2]; FERREIRA, B.A.[2]; DE ALMEIDA, R.P.[3]; TEIXEIRA, F.R.[1]; JEFFARES, D.C.[4]; CONSONNI, S.R.[2]; COELHO, A.C.[2]; MARUYAMA, S.R.C.[1].
1. DEPARTMENT OF GENETICS AND EVOLUTION, FEDERAL UNIVERSITY OF SÃO CARLOS, SP - BRA; 2. DEPARTMENT OF ANIMAL BIOLOGY, UNIVERSITY OF CAMPINAS, SP - BRA; 3. DEPARTMENT OF MEDICINE, FEDERAL UNIVERSITY OF SERGIPE, ARACAJU - SE - BRA; 4. DEPARTMENT OF BIOLOGY. UNIVERSITY OF YORK, GBR E-mail: sandrarcm@ufscar.br

Remoto Poster Session

Occurrence of monoxenous trypanosomatid infections in humans has been gradually increasing. We have shown that the majority of clinical isolates (CI) from visceral leishmaniasis in Sergipe, do not belong to *Leishmania* and are phylogenetically related to *Crithidia fasciculata*. We integrated genomic and phenotypic approaches to characterize this new parasite. For this, we performed whole-genome sequencing (WGS) of *Crithidia-like* LVH60a strain using Illumina and Oxford Nanopore. The genome was assembled in 36 chromosomes. Also, we performed WGS analysis of another 47 *Crithidia-like.* Read mapping rate of the 47 CI to *C. fasciculata* Cf-Cl genome was approximately 72%, whereas to *Crithidia-like* LVH60a was 99%. *In vitro* infection was performed using mice bone marrow-derived macrophages, J774 and THP-1 cell lines and parasite load analyzed 24h, 48h and 72h post infection. The average rate of infection for *Crithidia-like* was 27%, whereas for *L. infantum* HUUFS14 and *C. fasciculata* TCC039 strains were 40% and 5%, respectively. Cell growth at 25 °C showed similarity between *Crithidia-like* strains and TCC039, with a doubling-time of 9,6 and 7,81 h, respectively, whereas *L. infantum* doubling time was 15,2 h. Intertingly, cell growth at 35 °C showed similarity between *Crithidia-like* and *L. infantum* strains. Morphological analyses performed using scanning and transmission electron microscopy showed that *Crithidia*-like parasites are more similar to *C. fasciculata*. However, *L. infantum* had an average flagellar length nearly twice longer and greater cell body length and a smaller flagellar pocket when compared to *C. fasciculata* and *Crithidia*-like. These results demonstrated that *Crithidia-like* parasites presented an infectivity potential similar to the dixenous *L. infantum*, but the genomic and morphologic content is closer to a monoxenous *C. fasciculata*, showing that to study this new parasite will provide important insights into the evolution of parasitism in Trypanosomatidae. **Supported by:**FAPESP 2016/20258-0; FAPESP 2020/14011-8 **Keywords:**Crithidia;Leishmania infantum;visceral leishmaniasis.

PV-12 - **GENOMIC STUDY OF THE INTERMEDIATE METABOLIC PATHWAYS IN *Angomonas deanei*, A SYMBIONT-HARBORING TRYPANOSOMATID**

DOS SANTOS, L.M.L..
UFRJ- RJ - BRA.
E-mail: larissamaria@biof.ufrj.br

Some protozoa of the Trypanosomatidae family, as Angomonas deanei, maintain a mutualistic relationship with a symbiotic bacterium, representing an excellent model for studying the origin of organelles and cellular evolution. During the cell cycle, the bacterium maintains a close proximity with glycosomes, special types of peroxisomes that optimize ATP production by compartmentalizing most enzymes of the glycolytic pathway. Such organelles also participate in fatty acid oxidation, pyrimidine biosynthesis, carbon dioxide fixation, gluconeogenesis, succinate fermentation and contribute to the synthesis of compounds such as glycerol-3-phosphate. The present work evaluated in silico the influence of the symbiont on the intermediary metabolism of the host trypanosomatid and its possible role of integration with other cellular structures, to better understand the intense metabolic exchange that characterizes this relationship. To achieve this goal, we performed a genomic study of the glycolytic pathways, gluconeogenesis, pentoses, Krebs cycle, purine and pyrimidine metabolism of A. deanei and its symbiont, other trypanosomatids and prokaryotes served as comparative models. By aligning protein sequences, we observed that the symbiont is phylogenetically closer to prokaryotes, especially other trypanosomatid symbionts, than to its eukaryotic host. We also observed that in A. deanei all investigated pathways are practically complete, whereas the symbiont suffered gene loss in most synthesis routes, as those of energy metabolism. The symbiont has a very small, but highly functional genome, completing host pathways for the production of essential metabolites, as amino acids and vitamins. **Supported by:**CNPq, FAPERJ **Keywords:** endosymbiosis; trypanosomatid; intermediate metabolism.

PV-13 - **Cell cycle particularities in *Kentomonas*, a genus that contains symbiont-harboring trypanosomatids**

CAMELO, T.M.; GONCALVES, C.S.; DE SOUZA, W.; MOTTA, M.C.M..
UFRJ- RJ - BRA.
E-mail: taynacamelo@biof.ufrj.br

Kentomonas is a new genus of monoxenic trypanosomatids whose species host a bacterial symbiont in their cytoplasm. The β-Proteobacterium, also present in *Angomonas* and *Strigomonas*, maintains a mutualistic relationship with the protozoan, that is characterized by intense metabolic exchanges and ultrastructural changes. Furthermore, during the cell cycle, the bacterium division is coordinated with other host cell structures, so that each new cell contains a single symbiont. The main objective of this work is to analyze, from a structural point of view, aspects of the cell cycle in the *Kentomonas* genus, comparing the recently isolated *K. deaneorum* with *K. sorsogonicus* and also with *A. deanei* and *S. culicis*, the best characterized species. Results obtained by transmission electron microscopy and FIB-SEM showed that the *K. deaneorum* symbiont has a great proximity to the mitochondrial branches and that its kinetoplast has a disk shape, whereas in *K. sorsogonicus*, the symbiotic bacterium has an association with glycosomes and the kinetoplast has a trapezoidal format. In *A. deanei* and *S. culicis*, the symbiont shows proximity to glycosomes and also to mitochondrial branches. In all these species, the symbiont is close to the nucleus, indicating that the bacterium uses this organelle as a topological reference during the division process. It is important to mention that, curiously, *K. inusitatus* does not contain an endosymbiont, which may indicate that the symbiosis in *Kentomonas* is facultative and not mutualistic, as in the other genera. We conclude that *Kentomonas* species present intriguing particularities, constituting excellent models to study cell evolution and the diversity in trypanosomatids. **Supported by:**Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Código do Processo: 161032/2021-4 **Keywords:**endosymbiont-harboring trypanosomatids;Kentomonas genus;ultrastructural characterization of cell cycle.

PV-14 - **Location and expression of KAPS during Trypanosoma cruzi metacyclogenesis**

GONCALVES, C.S.[1]; FLOR, F.F.[1]; MELO, N.S.[1]; DE SOUZA, W.[1]; CAVALCANTI, D.P.[2]; MOTTA, M.C.M.[1].
1. UFRJ- RJ - BRA; 2. INSTITUTO NACIONAL DE METROLOGIA, QUALIDADE E TECNOLOGIA, RIO DE JANEIRO - RJ - BRA.
E-mail: florentinoflor17@gmail.com
2.

Trypanosoma cruzi has a complex life cycle, which assumes different forms of development during metacyclogenesis. During this process of cell differentiation, epimastigotes present in the insect gut transform into metacyclic trypomastigotes, which are capable of infecting vertebrate hosts. Important morphological and ultrastructural changes occur throughout the *T. cruzi* metacyclogenesis. In epimastigotes, the kinetoplast that contains the mitochondrial DNA (kDNA) has a disk shape, whereas in metacyclic trypomastigotes this structure becomes globular. Furthermore, the kDNA topology changes from highly condensed to a looser arrangement. These changes are related to the activity of small basic proteins, called Kinetoplast Associated Protein (KAPs). KAP4 and KAP7 are considered universal since they are present in all trypanosomatids so far analyzed. In this work we obtained protozoa which express KAPs fused to a fluorescent tag, thus allowing the evaluation of protein localization and expressions during metacyclogenesis. In epimastigotes, KAP4-mNG is located both in the kDNA network, in a semi-circle distribution, and in antipodal sites. In intermediate forms of the protozoan, which already present the globular kinetoplast, the same semi-circle location was observed, but the antipodal sites were not labeled. In metacyclic trypomastigotes, no labeling was observed for KAP4-mNG. In epimastigotes KAP7-mNG was observed punctually at the antipodal sites, while in intermediate forms of the protozoan and metacyclic trypomastigotes the labeling was not observed. Relative expression analysis showed that both KAPs were downregulated throughout metacyclogenesis, however this occurred in different ways. After nutritional stress, the expression of KAP4-mNG gradually decreased, while KAP7-mNG abruptly reduced and remained constant during the rest of the process. We conclude that the remodeling of the kDNA network is related to the negative regulation of universal KAPs. **Supported by:**CNPq and FAPERJ **Keywords:**Trypanosoma cruzi;metacyclogenesis;kinetoplast.

PV-15 - **Leishmanistatic in vitro activity of triclabendazole**

BORGES, B.S.; BUENO, G.D.P.; FIGUEIREDO, F.B.; DE MEDEIROS, L.C.A.S..
INSTITUTO CARLOS CHAGAS/FIOCRUZ-PR, CURITIBA - PR - BRA.
E-mail: beatrizsborges@hotmail.com

Leishmaniasis is a tropical neglected disease that causes approximately 1 million new cases and 30,000 deaths per year. Given the lack of adequate medication for the treatment of leishmaniasis, it is essential to use drug repositioning to save time and money in the search for new drugs, especially in the neglected disease scenario. Available treatments are still far from being fully effective in treating their clinical forms and are parenterally administered, which makes it difficult to carry out the complete treatment, in addition to being extremely toxic and, in some cases, leading to death. Triclabendazole (TCBZ) is a benzimidazole used in the oral treatment of fasciolosis in adults and children and presents lower toxicity in comparison with Amphotericin B (AmpB). When associated with the possibility of oral administration, it becomes a desirable candidate for the treatment of other parasitosis. The mechanism of action for TCBZ is not yet well understood, although it may have microtubules, polyamines, or ergosterol biosynthesis in the parasite as a pharmacological target. TCBZ has already shown antiproliferative activity against *T. cruzi*, *T. brucei,* and *L. infantum*. Within this context, this work aimed to evaluate the *in vitro* anti-*Leishmania* effects of TCBZ on *L. amazonensis* strain. The selectivity index (SI = CC_{50}/IC_{50}) was similar when compared with SI of AmpB. The evaluation of the cell cycle showed an increase of up to 10% of cells concentrated in S and G2, and morphological analysis by scanning microscopy showed high rates of dividing cells. Ultrastructural analyzes demonstrated large intracellular lipid concentrations, indicating alterations in lipid metabolism. Considering that TCBZ has the advantage of being cheaper and orally administrated, these results suggest that TCBZ is a promising candidate for use in the treatment of leishmaniasis. **Supported by:**CAPES E FIOCRUZ **Keywords:** LEISHMANIASIS; DRUG REPURPUSING; TRICLABENDAZOLE.

PV-16 - **Endosymbiosis in trypanosomatids: the coordinated division of the bacterium depends on the host microtubules' dynamism**

MORAES, J.R.; PRETA, C.M.C.C.; DE LIMA, L.S.G.; DE SOUZA, W.; ZUMA, A.A.; MOTTA, M.C.M..
INSTITUTO DE BIOFÍSICA CARLOS CHAGAS FILHO, RIO DE JANEIRO - RJ - BRA.
E-mail: santana.luann19@gmail.com

Some trypanosomatids, such as *Angomonas deanei*, co-evolve with a symbiotic bacterium in a mutualistic relationship. The bacterium presents coordinated division with other host cell structures, such as the nucleus and the kinetoplast, so that each protozoan contains a single symbiont. The use of microtubule polymerization inhibitors,as well as the RNAi system for tubulin, revealed that the symbiont division is cytoskeletal-dependent. In this work, Trichostatin A (TSA), an inhibitor of Histone Deacetylases (HDACs), was employed to verify the importance of microtubuledynamic instability for symbiont division. HDACs are enzymes that catalyze the removal of acetyl radicals (deacetylation) from proteins such as histones and tubulin, thus influencing the microtubule dynamism. The CRISPR-Cas9 system was also used to delete the HDAC6 gene, however, it was not possible to obtain null mutants for this protein, whichseems to be essential for *A. deanei* survival. The TSA treatment promoted inhibition of proliferation, but not of viability. Morphological and ultrastructural changes were observed in treated protozoa analyzed by different electron microscopy techniques and by fluorescence optical microscopy, such as symbiont filamentation and alterations in the cytoskeleton arrangement. Cell cycle arrest in the G1 phase was also observed. Similar structural changes were also reported in single allele-deleted mutants. In conclusion, the inhibition of deacetylation affects the division synchronicity of the symbiont with otherhost protozoan structures and that the bacterium division depends on the microtubule dynamism. **Supported by:**FAPERJ e CNPq **Keywords:** Symbiosis in trypanosomatids; coordinated division; microtubule cytoskeleton.

PV-17 - **Could the differentiation occur preferentially at some cell cycle stage of Trypanosoma cruzi?**

MENEZES, A.P.D.J.[1]; SILBER, A.M.[2]; DA CUNHA, J.P.C.[1]; SABBAGA, M.C.Q.B.E.[1].
1. INSTITUTO BUTANTAN, SÃO PAULO - SP - BRA; 2. USP - SP - BRA.
E-mail: anajmenezes@hotmail.com

The Trypanosoma cruzi is constantly submitted to the selective pressures that make them adapt to different host environments.The differentiation of epimastigotes into metacyclic trypomastigotes is triggered by cell starvation, pH, and temperature.The parasites change their morphology, metabolism, and gene expression.Many molecular mechanisms that could control differentiation,for example,the cell cycle,remain unknown.The cell cycle consists in a cascade of events that may interfere with how the parasites respond to the environmental stimulus.We aim to verify if the differentiation trigger occurs preferentially in a specific cell cycle stage of T.cruzi.We synchronized exponential epimastigote cultures (CL Brener) in different cell cycle stages using hydroxyurea (HU).Immediately after the drug release,most parasites were in G1/S transition. After four and eight the parasites were in S and G2/M, respectively. We confirmed the cell synchronization by submitting the parasite samples to flow cytometry.We also used exponential and stationary epimastigotes without HU treatment as a control.Following the cell cycle synchronization,we submitted the parasites to in vitro differentiation.Our findings suggest a higher number of metacyclic trypomastigotes in G1/S and G2/M synchronous culture when compared to those submitted to differentiation in the S stage.Interestingly, the culture supernatant and adhered parasites were mostly in G1/S no matter what cell cycle stage the differentiation stimulus was given.The differentiation in the exponential phase was enriched in intermediated parasites on the culture supernatant.In contrast,the stationary phase culture has had only undifferentiated epimastigotes and metacyclic trypomastigotes.These preliminary results have shown that parasites in G1/S and G2/M phases could be more responsive to differentiation stimuli.We are now researching other aspects of these intriguing molecular mechanisms that connect the cell cycle to differentiation. **Supported by:**Fundação de Amparo a Pesquisa do Estado de São Paulo / 2019/21354-1 **Keywords:**Trypanosoma cruzi; Differentiation; Cell cycle.

PV-18 - **Study of the role of Multicopper Oxidases genes in first stage nymphs of *Rhodnius prolixus* infected with *Trypanosoma cruzi*.**

GONÇALVES, K.C.D.S.; OKUDA, L.V.O.; DIAS, F.D.A.; CONTRERAS, H.D.P.; DE OLIVEIRA, P.L..
UNIVERSIDADE FEDERAL DO RIO DE JANEIRO - UFRJ, RIO DE JANEIRO - RJ - BRA.
E-mail: keylacristiny@gmail.com

Multicopper oxidase (MCO) are a family of copper-containing oxidases that in insect have the functions associated to essential physiological process including cuticle sclerotization, iron metabolism and immunology. However, the participation of MCO in parasite-insect interactions has notbeen deeply studied. Through *in silico* analysis of gut transcriptome of first instar nymphs of *Rhodnius prolixus*, a vector of *T. cruzi*, we identified the presence of 11 genes in members of the families of MCO. Of these genes, 2 genes RPRC 002327 and RPRC000040 showedsignificantly increased expression after infection with the *T. cruzi*. Through the qPCR analyses, we confirmed that the gene RPRC000040, here denominated rpMCO2b, had a significant expression increase when infected with different parasite loads with trypomastigote stages of the parasite. Interestingly, comparison of specific relative expression of different organs showed the MCO2b is expressed in all organs excepted heart, with the highest levels of expression found in the posterior midgut, anterior and rectum followed by the testicles and fat body. MCO2b knockout did not change the course of hemoglobin digestion or embryogenesis, but resulted in a ten-fold increase in the size of resident microbiota. This result suggests the involvement of MC2b in midgut immune response in *R. prolixus*.
Supported by:CNPQ **Keywords:**Multicopper oxidase; Rhodnius prolixus;Trypanosoma cruzi.

PV-19 - **Metabolic enzymes in the nuclear compartment: a putative link with epigenetic changes in *Trypanosoma cruzi***

DE CASTRO, C.G.[1]; SILVA, H.G.D.S.[1]; MARCHESE, L.[2]; ALENCAR, M.B.[2]; SABBAGA, M.C.Q.B.E.[3]; SILBER, A.M.[2]; DA CUNHA, J.P.C.[1].
1. INSTITUTO BUTANTAN, SÃO PAULO - SP - BRA; 2. USP - SP - BRA; 3. INSTITUTO BUATANTAN, SÃO PAULO - SP - BRA.
E-mail: camilagachet@gmail.com

Studies associating epigenetics and gene expression are already well known in many organisms. However, this connection is not so evident in Trypanosomes, whose gene expression regulation is based on post-transcriptional mechanisms. Compared to other eukaryotes, Trypanosomatids harbor peculiarities both in their epigenetic and metabolic programs. Different environments make the availability and use of metabolites very different in each life stage of the *Trypanosoma cruz*i parasite, which transits between invertebrate and mammalian hosts during its cycle. Therefore, we aimed to delve deeper into the relationship between epigenetics and metabolism searching metabolic proteins enriched in chromatin and nuclear proteomics public datasets. Specially enzymes related to the metabolism of acetyl-CoA, α-ketoglutarate, and succinyl-CoA, that are important for the acetylation, methylation, and succinylation processes, respectively. We found that 16 enzymes belonging to glycosome, mitochondria, and cytoplasm involved in glycolysis, TCA cycle, and SAM metabolism were found in the nuclear compartment and associated with chromatin in *T. cruzi*. Using the Tryp Tag platform, we found that 35,3% of these proteins in *Trypanosoma brucei* have nuclear localization using the neon green tag. To confirm the nuclear localization of these enzymes, we are currently performing activity measurements with epimastigote nuclear extracts. Preliminary analysis confirms nuclear hexokinase activity, while the pyruvate kinase (negative control) activity was not detected in the nuclear extracts. We choose six enzymes found in the proteomes to evaluate their enzymatic activity. In addition, we are currently using CRISPR-Cas9 to tag these selected metabolic targets to confirm their nuclear location. We intend to confirm the location and respective activities of these selected metabolic enzymes in the nucleus, showing a new interplay between metabolism and histones post-translational modification in *T. cruzi.* **Supported by:**FAPESP 2021/12469-0 **Keywords:**Trypanosoma cruzi; metabolism;epigenetic.

PV-20 - **Oxidative stress response mechanisms in phosphorylation of eIF2α and AMPK in T. cruzi**

COSTA, M.A.V.; SILVA, M.A.; SCHENKMAN, S..
UNIFESP, SÃO PAULO - SP - BRA.
E-mail: miiih.costa@hotmail.com

Trypanosoma cruzi epimastigotes that proliferate in the gut of triatomines undergo metacyclogenesis to become infective to mammalian host. This occurs through external stimuli mediated by different types of stress, including nutritional and oxidative stress. In eukaryotes, specific protein kinases are activated and phosphorylate the eukaryotic translation initiation factor (eIF2) which causes translation repression with the activation of stress response factors. AMPK (AMP-activated protein kinase) also acts as an energy sensor in cells that is activated by AMP/ADP increase through phosphorylation of a conserved threonine residue, resulting in several catabolic processes to regenerate ATP. To verify the role of these two pathways in T. cruzi, we obtained eIF2α-mutated parasites from Y and DM28c strains, in which the phosphorylated residue of eIF2α was replaced by alanine using CRISPR-Cas9. Y-strain parasites became highly susceptible while DM28c was less affected by hydrogen peroxide-induced stress. Oxidative stress induced AMPK phosphorylation in wild type eIF2α of both strains. In contrast, AMPK is more phosphorylated in eIF2α mutant of Y than of the DM28c strain. We also found higher levels of in oxidative species in the mutant of Y strain than in DM28c. These results indicate that eIF2α phosphorylation induces oxidative stress responses that can prevent activation of AMPK. Therefore, both pathways might be relevant for parasite differentiation into infective forms. **Supported by:**Fapesp - 2021/12515-1 **Keywords:**eIF2;AMPK;stress reponse.

PV-21 - **The tDNA chromatin changes positively correlate with transfer RNA expression levels in *Trypanosoma cruzi* life forms.**

SILVA, H.G.D.S.[1]; DE LIMA, P.L.C.[1]; RÓSON, J.N.[1]; CALDERANO, S.G.[2]; DA CUNHA, J.P.C.[2].
1. UNIVERSIDADE FEDERAL DE SÃO PAULO E LABORATÓRIO DE CICLO CELULAR DO INSTITUTO BUTANTAN, SP - BRA; 2. LABORATÓRIO DE CICLO CELULAR DO INSTITUTO BUTANTAN, SP - BRA.
E-mail: herbert.silva@esib.butantan.gov.br

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 associated with tDNAs are more enriched in epimastigote (Epi) than in metacyclic trypomastigote (MT) forms. Interestingly, during the differentiation of Epi to MT life forms, a decrease in their translatome and transcriptome occurs. 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 important to regulate tRNA expression and to determine the moment, during metacyclogenesis, that tDNA's chromatin became closed. Thus, we applied global nascent RNA transcriptome (Gro-seq) obtained in Epi forms in association with mature tRNA expression analysis by the northern blot in different life forms of *T. cruzi*. The tDNA chromatin changes were monitored by FAIRE-qPCR of three tDNA loci during the metacyclogenesis. The results showed a positive correlation between open tDNA chromatin and tRNA nascent expression levels in Epi. We also verified that the Epi has about two times more mature tRNA transcripts than MT. Furthermore, tDNA's chromatin became closed within 48 hours of metacyclogenesis induction. Studies are in progress to determine whether the closed tDNA chromatin is an important step to decrease transcription and translation in MT forms or a consequence of other events not yet fully understood. **Supported by:**FAPESP 21/11419-9 **Keywords:**Trypanosoma cruzi;tRNA expression;cell differentiation; .

PV-22 - **Could Hi-C data analysis indicate putative *Trypanosoma cruzi* centromeric regions?**

BELLINI, N.K.; SILVA, K.D.S.E.; BARBOSA, J.G.C.; KUHNE, R.D.T.; PIRES, D.D.S.; DA CUNHA, J.P.C..
CELL CYCLE LABORATORY, BUTANTAN INSITUTE, SÃO PAULO - SP - BRA.
E-mail: natalia.bellini@esib.butantan.gov.br

The genome organization of trypanosomatids is peculiar due to the lack of classical RNA Pol II promoters regions; thereby, post-transcriptional mechanisms are the main strategy for regulating gene expression. Coding protein genes are transcribed as polycistronic units, and little is known about their disposal and composition in centromeric regions. Recent literature pointed out the chromatin tridimensional organization as a new layer of epigenetic regulation and highlight chromosomes grouped in nuclear territories, and centromeric DNAs often in close contact in the nucleus. Centromeres of only three chromosomes in *T. cruzi* have been identified so far. We are studying the role of chromatin topology in gene expression, the chromosomal regions more likely to interact spatially, and the putative centromeres in *T.cruzi* Brazil A4 by using genome-wide chromosome conformation capture (HiC) public data. We identified active and inactive compartments, topologically associated domains (TADs), and DNA loops. The first principal component analysis (PCA) plots can reflect the rough structure of chromosomes, dividing them on the chromosome arms and revealing the centromeric positions. Our results suggest eleven chromosomes with PCA1 typical of chromosome arms. Functional genomic analysis (e.g., GO and REVIGO) reveal lesser functional pathways heterogeneity for these centromeric regions. We do not observe significant differences in the number of genes on putative centromeric segments compared to their juxtaposing areas. We performed BLASTn searches using the retroelements VIPER and SIRE, and the repetitive regions TRS of centromeres from other *T.cruzi* and *T.brucei* strains. We also developed bash scripts to process the BLASTn results and filter the best matches. We observed the spatial interaction of centromeres in the nucleus is also true for *T. cruzi.* Our work reinforces the Hi-C relevance for exploring centromeres and expands our knowledge of genome organization on trypanosomes. **Supported by:**FAPESP, Projects 2021/03219-0 and 2018/15553-9 **Keywords:**high throughput chromatin conformation capture;centromere;Trypanosoma cruzi.

PV-23 - **Characterization of TgSRS12B, a surface protein predominant in Brazilian isolates of Toxoplasma gondii.**

FARIAS, M.A.[1]; SEVERO, V.R.[1]; FRAGOSO, M.S.I.[1]; DE SIQUEIRA, C.D.M.[1]; FERRARINI, M.G.[1]; AVILA, A.R.[1]; DUARTE, E.D.S.M.[2]; NARDELLI, S.C.[1].
1. FIOCRUZ-PR, CURITIBA - PR - BRA; 2. UFMG, MG - BRA.
E-mail: melissafarias95alves@gmail.com

Toxoplasma gondii is the etiological agent of toxoplasmosis that affects ¼ of the world population. Toxoplasmosis is very dangerous in immunosuppressed patients, such as HIV patients, transplanted patients, or pregnant women. In Brazil, the high genetic variability makes these “atypical strains" cause more severe symptoms compared to North Hemisphere strains. Toxoplasma is part of the Apicomplexa phylum, characterized by the Apical complex, releasing several proteins related to invasion, parasitophorous vacuole formation, and maintenance. However, the first step of invasion is the adhesion to the cell host surface. One family protein that participates in that step is the SAGs. More than a hundred SAGs are annotated, and anchored by GPI, but few are characterized. The most characterized is the SAG1, the most abundant in reference strains and a promising candidate for diagnostic and vaccines. We analyzed the surface proteins of 5 isolates and references strains by mass spectrometry to understand the Brazilian strains. We identified more than 20 SAGs, and several transmembrane proteins still not identified. We confirmed that SAG1 is the most expressed SAG also in Brazilian strains. Most importantly, we identified some SAGs that are differentially expressed in Brazilian strains compared with the reference strains. One of these was the SRS12B, present in all 5 Brazilian strains but not in the reference strains RH and ME49. In order to study SRS12B, we are performing overexpression and soon knockout using CRISPR technology to understand the role of this SAG in the infection. It is important to notice that most SAGs that are differentially expressed are more present in bradyzoites (ToxoDB), and we did not induce differentiation. However, we noted that some Brazilian strains have a spontaneous differentiation by immunofluorescence. Understanding these specific Brazilian strains can reveal some proteins that can improve or better diagnose toxoplasmosis in Brazil. **Supported by:**Fiocruz, Capes, CNPq e Fundação Araucária **Keywords:**Toxoplasma gondii;Diagnosis;SAGs.

PV-24 - **Effects of meiosis-related genes deletion in Leishmania hybridization**

PRETA, C.M.C.C.; FERREIRA, T.R.; SACKS, D..
NATIONAL INSTITUTES OF HEALTH - NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES, EUA.
E-mail: carol.cattapreta@gmail.com

Clinical outcomes of leishmaniasis are known to be related to *Leishmania* species diversity and the emergence of new strains. Previously, a cryptic sexual cycle has been described involving promastigote stages developing in the sand fly vector. The generation of hybrids was demonstrated in laboratory during sand fly infections and, more recently, in culture after DNA damage. We previously identified a subgroup of DNA stressed cells that upregulated a number of meiosis-related genes. Here, we used *L. tropica* parental strains MA37 and L747 that have a high mating efficiency to generate CRISPR-Cas9 competent cell lines, to delete the meiosis-related genes HAP2-1, HAP2-2, SPO11, MND1, DMC1, HOP1 and HOP2, and investigate their respective roles in genetic exchange. We were able to generate null mutants for each of these genes in both strains by substituting the whole CDS for Puromycin N-acetyltransferase (PAC) gene. For *in vitro* crossings, we used the null mutants from one of the strains in combination with a control line containing the Blasticidin S deaminase (BSD) gene integrated into the SSU rRNA locus, and selected for cells resistant to both PAC and BSD. For null mutants of *L. tropica* MA37, two null mutants showed a significant decrease in the minimum frequency of hybridization-competent cells: DHAP2-2 (2.2-fold lower, $p = 0.0021$) and DHOP1 (no hybrids recovered). By contrast, L747 null mutants showed a reduction for DHAP2-2 (1.5-fold lower, $p < 0.0001$), DDMC1 (1.9-fold lower, $p < 0.0001$), DHOP2 (5.5-fold lower, $p=0.0003$) and DHOP1(no hybrids recovered). *Lutzomyia longipalpis* infections indicated that the *L. tropica* L747 DHOP1 mutant was impaired for hybridization *in vivo.* These findings implicate the involvement of protein components of the meiotic machinery in *Leishmania* hybridization. Further experiments, including the generation and testing of re-expressor lines, are being performed to further investigate the role of meiosis-related genes in genetic exchange. **Supported by:**National Institutes of Health **Keywords:**Leishmania;Sexual cycle;Meiosis.

PV-25 - **Translation initiation factors EIF4E3 and EIF4E4 associate to different proteins and mRNAs targets in *Trypanosoma cruzi*.**

GABIATTI, B.P.; FREIRE, E.R.; HOLETZ, F.B..
ICC-FIOCRUZ/PR, CURITIBA - PR - BRA.
E-mail: freireer@hotmail.com

In Trypanosomatids the control of gene expression is mainly performed by post transcriptional mechanisms and the total mRNA levels not always are according to corresponding protein levels, indicating a specific mRNA selection. Particularly, translation initiation seems to be regulated in trypanosomes, performed by several eIF4F-like complexes, whose roles in translation regulation have not being fully elucidated. When exponentially growing *T. cruzi* cells are subjected to nutritional/pH stress, a global repression of translation occurs, but a few transcripts are still associated to ribosomes. Here we investigated the two eIF4E paralogues, EIF4E3 and EIF4E4, in exponentially growing and stressed cultures by immunoprecipitation assays and analyzing the protein partners and the associated mRNAs. The results have shown that EIF4E3 forms a complex with eIF4G4 and both PABP1 and PABP2. On the other hand, eIF4E4 forms a complex with eIF4G3, eIF4A1 and PABP1. Both complexes are resistant to RNAse A treatment, suggesting they are formed by direct interactions. Several other translation initiation factors were found associated with EIF4E3 complex, indicating a role on translation, while eIF4E4 was mostly found with other RNA binding proteins. Both eIF4F complexes proved to be stable in stress condition, indication that complex composition was not the mechanism of the global repression of translation. However, some interactions are absent or reduced in stressed cells. Transcriptome analysis for both complexes have shown that EIF4E3 complex associates with several transcripts' classes. Alternatively, eIF4E4 associates preferably to ribosomal protein mRNAs. Surprisingly, under stress conditions a seemly higher abundance of mRNAs was captured, but composed to similar transcripts types for both factors, suggesting that the eIF4F-mRNA interaction was more stable. The data acquired indicates that both eIF4F complexes seem to have complementary roles in translation in *T. cruzi*. **Keywords:**T; cruzi;Translation Initiation;eIF4E.

PV-26 - **Characterization of *Giardia intestinalis* cytoskeleton: A new extra-axonemal structure evidenced by high resolution microscopy**

CURTI, R.V.[1]; GADELHA, A.P.R.[2]; DE MIRANDA, K.R.[3]; BENCHIMOL, M.[4]. 1. 1LAB. DE ULTRAESTRUTURA CELULAR HERTHA MEYER, IBCCF, UFRJ, RJ, BRAZIL, RJ - BRA; 2. 2LAB. DE MICROSCOPIA APLICADA ÀS CIÊNCIAS DA VIDA, DIMAV - INMETRO, RJ, - RJ - BRA; 3. INSTITUTO NACIONAL DE CIÊNCIA E TECNOLOGIA EM BIOLOGIA ESTRUTURAL E BIOIMAGENS, AND CENTRO NACIONAL, RJ - BRA; 4. UNIVERSIDADE DO GRANDE RIO , RJ, BRAZIL, RJ - BRA.
E-mail: raphaelverdan@gmail.com

Giardia intestinalis is an intestinal protozoan parasite that causes a diarrheal disease named giardiasis. During its life cycle, this protozoan has two morphologically distinct developmental stages: the cyst, which is the infective form, and the trophozoite responsible for colonization of the host intestine. *Giardia* cytoskeletal is composed primarily of microtubules organized in unique structures that are involved in a wide range of functions as cell motility and adhesion. *Giardia* flagella, displayed the 9:2 + 2 microtubular pattern, distributed in: anterior, posteriolateral, ventral and caudal flagella. The flagellar pairs differ in length, position, and their association with specific structures. The marginal plate and dense rods are associated with the axoneme of the anterior flagella and funis is associated with the axoneme of the caudal flagella. However, the composition and function of these axoneme-associated structures are unknown. In this work we analyze the externalization region of axonemes of the G. intestinalis and characterized an associated structure using high resolution microscopy techniques. Cells are treated with 2% NP-40 detergent for 10 min to remove the plasma membrane and expose the cytoskeleton. Then, the cells are observed by scanning electron microscopy, atomic force microscopy and transmission electron microscopy. Our observations in no-extracted and extracted membrane cells show that the externalizing region of the flagellum presents a differentiated morphological domain. Negative stain reveals an extra-axonemal structure that is observed associated with all pair of trophozoite flagella. A new extra-axonemal structure is around the axonemes and measures about 50 nm in thickness. This structure remains attached to the axoneme even when the connections between the axoneme microtubules are disrupted after detergent treatment. Our data will contribute to a better understanding of the organization and functional role of the *Giardia* flagella. **Supported by:** CAPES and FAPERJ (88887.512242/2020-00; E-26/202.824/2017). **Keywords:**Giardia intestinalis;high resolution microscopy;G; intestinalis cytoskeleton.

PV-27 - **Biochemical characterization of CRL (Cullin RING ligases)- like E3 ubiquitin ligases in Leishmania infantum**

DE CORREIA, C.R.S.T.B.[1]; MARUYAMA, S.R.C.[2]; COELHO, A.C.[3]; ROGERIO, L.[2]; TAKAMIYA, N.T.[2]; RODRIGUEZ, G.M.[2]; TEIXEIRA, F.R.[2].
1. FACULDADE DE MEDICINA DE RIBEIRÃO PRETO, USP, SP - BRA; 2. UNIVERSIDADE FEDERAL DE SÃO CARLOS, SP - BRA; 3. UNIVERSIDADE ESTADUAL DE CAMPINAS, SP - BRA.
E-mail: rolemberg.camila1@gmail.com

The ubiquitin proteasome system (UPS) is responsible for the most of intracellular proteolysis in eukaryotes and the ubiquitination process occurs through the action of three enzymes: E1 (ubiquitin-activating enzyme), E2 (ubiquitin-carrying enzyme) and E3 (ubiquitin-ligases) that play a key role in this process, recognizing and transferring ubiquitin to its substrate. In parasitic protozoan, intracellular proteolysis is essential for the alternation of hosts in their life cycles and consequently for the success of parasitism. The *Leishmania* proteasome has a high identity to that of humans, being considered a target for treatment of leishmaniasis, however little is known about UPS in *Leishmania* genus. We aim to characterize the *Leishmania infantum* orthologous to the human genes SKP1, RBX1 and CUL1 respectively, which are components of the CRLs in humans. We evaluate through immunoprecipitation in mammalian HEK293T cells the interaction of the human components of CRLs and the *L.infantum* orthologs. Human F-box protein 7 (FBXO7) interacted with SKP1 from *L.infantum* and human Cullin1 interacted with SKP1 and RBX from *L.infantum*, suggesting that the regions responsible for the interaction among these proteins are conserved in the parasite proteins, suggesting the presence of the CRL complex in *L.infantum*. The parasite was genetically modified through the CRISPR-Cas9 strategy to generate *L. infantum* Cas9T7 lineages hygromycin resistant and it showed the same morphology and growth as the wild-type. After that, *L. infantum* Cas9T7 was used to produce *L. infantum* lineages with SKP1, Cullin 1 or RBX in fusion with 3xmyc-mCherry. These lineages will be used to develop immunoprecipitation of the parasites lysates with anti-myc resin aiming the identification of the CRLs components partners in *L.infantum* throught mass spectrometry. Our interaction results indicates a conservation of CRLs components in *L.infantum*, however their partners were not identified yet. **Supported by:**CNPq **Keywords:**Leishmania;Ubiquitin proteasome system (UPS);E3 ubiquitin-ligase.

PV-28 - **Localization of the protein myosin C (TcMyoC) along the life cycle of *Trypanosoma cruzi*.**

JÚNIOR, M.V.A.D.; ALCANTARA, C.D.L.; DE SOUZA, W.; DA CUNHA-E-SILVA, N.L..
INSTITUTO DE BIOFÍSICA CARLOS CHAGAS FILHO (IBCCF/UFRJ), RIO DE JANEIRO - RJ - BRA.
E-mail: marcusvinicius.dantas@outlook.com

Myosins constitute a superfamily of motor proteins capable of moving on actin filaments, involved in several functions including endocytosis. Besides Myosin 1, conserved in several eukaryotes and Myosin XIII class, particular to kinetoplastids, *Trypanosoma cruzi* has 8 myosin genes (TcMyoA to TcMyoH-d), which arose from the expansion of Myosin XIII class. Due to the absence of these expanded genes in *L. major* and *T. brucei*, an association with the endocytic pathway was suggested. Recently, the participation of MyoF in the endocytic pathway of *T. cruzi* was demonstrated. Aiming at identifying the participation of TcMyoC in the endocytic traffic, we generated a mutant strain fusing the mNeonGreen (mNG) tag gene and 3 c-myc sequences to the N- terminal region of the *TcMyoC* gene by CRISPR-Cas9. Tagged epimastigotes observed alive showed TcMyoC close to the preoral ridge region. Endocytosis assays with transferrin-CF555 under different conditions, showed that TcMyoC followed the tracer, from the uptake at the cytostome-cytopharynx complex to the lysosomes., After detergent fractionation and Western blotting with anti-myc, TcMyoC was found mostly, but not exclusively, in the insoluble fraction, suggesting an association with the cytoskeleton. Immunogold labelling, with anti-mNG antibody, in negative stained cytoskeleton preparations, showed TcMyoC at the anterior region of the cytopharynx microtubules, at the preoral ridge. During metacyclogenesis, we found TcMyoC protein at the posterior region of stressed epimastigotes and intermediate forms. Metacyclic trypomastigotes were devoid of mNG::TcMyoC signal. After infection of mammalian cells, TcMyoC was found along the cytostome-cytopharynx complex of intracellular amastigotes. Extracellular amastigotes showed a punctual signal at the anterior region and trypomastigotes from the culture supernatant presented no signal. These results strongly suggest the close association of TcMyoC to the endocytic pathway of *T. cruzi*. **Supported by:**CNPq e 161037/2021-6
Keywords: TcMyoC;actin cytoskeleton;Trypanosoma cruzi.

PV-29 - **Production of *Trypanosoma cruzi* lineages expressing a panel of mutant versions of the TcRlp Ras-like GTPase.**

DE FARIAS, K.L.; NEPOMUCENO-SILVA, J.L.; VALÉRIO, C.D.D.; AVELINO, B.K.A..
UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RJ - BRA.
E-mail: agriasknt@gmail.com

Ras family GTPases are molecular switches that regulate cellular proliferation and differentiation by cycling between GTP and GDP-bound states. Ras family proteins also undergo isoprenylation (geranylgeranylation or farnesylation) on a c-terminal CaaX motif that enables membrane association, which is essential for their activity. The parasite *Trypanosoma cruzi* has only one Ras gene, which encodes the protein TcRlp (*T. cruzi* Ras-like protein), whose function is unknown. TcRlp has the CaaX motif CVLL, which is a target to geranylgeranylation. Our aim is to start the characterization of the TcRlp protein. In order to investigate the ability of TcRlp to bind and hydrolyze GTP, we have produced 3D homology-based models of wild-type TcRlp, as well as of mutants predicted to have impaired GTPase activity (TcRlp-G12V and Q61K – positive dominants) or increased affinity for GDP (TcRlp-S17N – negative dominant), and docked them *in silico* with GDP and GTP. The Q61K and S17N mutants presented molecular interactions which are consistent with positive and negative dominants, respectively. Next, we have produced mutants of the TcRlp gene, cloned them in the pTEX-GFP vector and transfected into Dm28c epimastigotes. Thus, the strains GFP-TcRlp-WT (wild-type), GFP-TcRlp-G12V, GFP-TcRlp-Q61K, GFP-TcRlp-S17N, GFP-TcRlp-dCaaX (non-isoprenylated) and GFP-TcRlp-CQLF (a farnesylated mutant), as well as the double mutants GFP-TcRlp-Q61K-dCaaX, GFP-TcRlp-Q61K-CQLF and GFP-TcRlp-G12V-CQLF were successfully produced. The intracellular localization of mutant proteins was investigated by fluorescence microscopy. TcRlp-WT and Q61K displayed a cytoplasmic-vesicular distribution, while TcRlp-G12V oscillated between cytoplasm and nuclei. TcRlp-S17N, -CQLF, -Q61K-CQLF and -G12V-CQLF all presented nuclear localization, and TcRlp-dCaaX and -Q61K-dCaaX were dispersed in cytoplasm. As selected mutations affected TcRlp localization, these strains will be valuable to investigate the cellular role of TcRlp. **Supported by:**PIBIC-UFRJ **Keywords:**Ras family GTPases;Trypanosoma cruzi;Protein prenylation.

PV-30 - **Characterization of a Fatty-Acid Binding Protein-like in *Leishmania amazonensis***

GOMES, M.A.M.[1]; BOY, R.L.[1]; FLOETER-WINTER, L.[1]; SALINAS, R.K.[2]; LARANJEIRA-SILVA, M.F.[1].
1. INSTITUTO DE BIOCIÊNCIAS DA USP - SP - BRA; 2. INSTITUTO DE QUÍMICA DA USP - SP - BRA.
E-mail: marcoantoniomg90@gmail.com

Leishmania parasites are the causative agents of a group of diseases collectively known as leishmaniases. These neglected tropical diseases are endemic in 98 countries, where more than 1 billion people are at risk of infection. There are no vaccines and therapy remain limited due to drug toxicity and parasite's resistance. During their life cycle, *Leishmania* alternates between invertebrate and vertebrate hosts. Hence, they must adapt to different environments and compete with their hosts for several essential nutrients, such as lipids and fatty acids (FAs). Considering the crucial role of Fatty-Acid Binding Proteins (FABPs) in lipid metabolism, we looked for proteins containing FABP-like domains in the *Leishmania* genome. We identified a putative *L. mexicana* gene (LeiFABP) encoding a conserved hypothetical FABP-like domain. Therefore, our goal is to functionally characterize LeiFABP in *L. amazonensis*. We cloned constructs of LeiFABP fused to GFP in the *Leishmania* expression plasmids pXG-GFP+ and pXG-GFP2+. With these, we have been investigating the subcellular localization of LeiFABP in *L. amazonensis*, and characterizing LeiFABP overexpressing parasites regarding replication and virulence. We observed that LeiFABP expression is differentially modulated during in vitro growth of *L. amazonensis* promastigotes. LeiFABP characterization may help elucidate the distinctive features of *Leishmania*' FAs metabolism and trafficking, which may help to uncover new targets for the development of better therapeutic strategies against leishmaniases. **Supported by:**FAPESP Nº de Processo 2020/07465-2 **Keywords:** Leishmaniasis;lipid metabolism; neglected tropical diseases.

PV-31 - **Comparative genomic analysis of two *Leishmania Infantum* Brazilian strains: MHOM/BR/74/PP75 and HUUFS14.**

ROGERIO, L.A.[1]; TAKAHASHI, T.Y.[1]; TAKAMIYA, N.T.[1]; TORRES, C.[1]; DOS SANTOS, Y.O.C.[1]; SERAFIM, T.D.[2]; RIBEIRO, J.M.[2]; DE ALMEIDA, R.P.[3]; MARUYAMA, S.R.C.[1].
1. UNIVERSIDADE FEDERAL DE SÃO CARLOS, SP - BRA; 2. NATIONAL INSTITUTES OF HEALTH-EUA; 3. UNIVERSIDADE FEDERAL DE SERGIPE, SP - BRA.
E-mail: yasmin.santos@estudante.ufscar.br

Visceral leishmaniasis (VL) is defined as a non-contagious infectious disease caused by the protozoan of the genus Leishmania. Comparative genomic analyses compare the composition of genomes, e.g. presence or absence of genes, gene organization, gene duplication, genetic variations and relate these findings to the functional characteristics of the species analyzed. Here, our aim was to perform a comparative genomic analysis between two L. infantum Brazilian strains with the JPCM5 reference strain (Spanish). For this study, two Brazilian strains were analyzed: HUUFS14 isolated in 2009 from a VL patient in Sergipe and MHOM/BR/74/PP75, isolated in 1974 from a child in Bahia. Genomic DNA samples of the MHOM/BR/74/PP75 and HUUFS14 were sequenced in Illumina platform (MHOM/BR/74/PP75: 2x150bp, 490x, 6.2Gb; HUUFS14: 2x 150bp, 150x, 4.3Gb). Genomes were assembled using the Companion software. The total number of genes, protein coding genes, pseudogenes and ncRNA were analyzed and compared. The copy number variation (CNV) was performed in Artemis BamView software by mapping the paired-end reads in the JPCM5 reference genome. Subsequently, the genes were functionally annotated as type of gene and metabolic pathways using TriTrypDB. Genomic analyses showed a decrease in genes, protein coding and ncRNA number of MHOM/BR/74/PP75. However, the increase in the number of pseudogenes of both strains is remarkable when compared to JPCM5. In total, 332 genes had a decrease in CNV, in which 302 were protein-coding genes, 28 ncRNA and 2 pseudogenes. Interestingly, the genes with the greatest decreases in CNV observed were: Amastin-like (n=31), Elongation factor (n=15) and GP63 (n=7). We found 288 metabolic pathways, with purine metabolism the pathway with the highest single gene count (n=27), followed by the cysteine biosynthesis pathway (n=16). Genome annotation of Brazilian strains is important for comparative genomics of clinical isolates obtained from VL patients in Brazil. **Supported by:**FAPESP- IC: 2021/14615-3 ; FAPESP- JP: 2016/20258-0 **Keywords:** Leishmania Infantum; comparative genomic; reference genome.

PV-32 - **Pseudopod projection and modulation in the testate amoeba *Arcella intermedia* (Arcellinida:Amoebozoa)**

DA SILVA, T.F.; KULESSA, L.S.; LAHR, D.J.G.. USP - SP - BRA.
E-mail: taina.figueiredo.silva@usp.br

Testate amoebae are unicellular eukaryotes that have a shell (test) covering the organism's cell. These organisms use projections of their cytoplasm called pseudopods for locomotion, feeding, and other complex behaviors. The pseudopod is considered a key morphological character, and it is vastly used to describe, distinguish, and classify amoebae groups. Despite this pivotal 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. Also, the correlation between this variability and the type of substrate on which the organism locomotes is unknown. In this preliminary work, we used the testate amoeba specie *Arcella intermedia* (Arcellinida:Amoebozoa) in three different types of substrate (i.e., agar plates, plastic bottles and glass plates with liquid media) to investigate i) the morphological variability range regarding the organism performance (i.e., locomotion speed), ii) whether the shell attributes and distinct types of substrate correlate to the performance, iii) in case correlations are found, describe how the correlation is given and its relations among the variables.
Thus, we measured the shell diameter and area, pseudopod number per individual, and pseudopod length and width of 30 individuals from each substrate. We computed the cell's measurements and the locomotion speed as variables in generalized linear models, known as GLM, for further analysis, building a model for each type of substrate.
The results showed that the locomotion speed average is very similar among the three substrates and revealed a negative correlation between the locomotion speed and the number of pseudopods. The average number of pseudopods was noticeably higher in individuals of agar plates. The previous results suggest that these individuals can modulate their pseudopods according to the substrate, actively managing to sustain a performance rate. **Supported by:**CAPES-PROEX - 88887.659012/2021-00 **Keywords:**cellular motility;microeukaryotic plasticity;variability.

PV-33 - **Study of phospholipases A_2 in *Rhodnius prolixus* midgut during *Trypanosoma cruzi* infection**

DE ARAUJO, M.F.C.; CARDOSO, L.S.; KLUCK, G.E.G.; ATELLA, G..
UFRJ- RJ - BRA.
E-mail: mfaraujo@bioqmed.ufrj.br

Rhodnius prolixus is a hematophagous insect known to be a vector of *Trypanosoma cruzi*, etiologic agent of Chagas's disease. Fighting against the vector is one way to prevent this disease. Finding insect vectors' molecules that are crucial for the infection is necessary for the control of this disease. In this scenario, previous studies from our group demonstrated the involvement of lysophosphatidylcholine (LPC), in different aspects of the infection. Thus, the aim of this work is to study a superfamily of enzymes that can promote the release of this lysophospholipid (LPL), phospholipases A_2 (PLA_2). These enzymes can catalyze the hydrolysis of glycerophospholipids in the *sn-2* position, releasing fatty acids and LPLs. In the present study, we investigated whether the infection by *T. cruzi* modulates the gene expression of secreted PLA_2 ($sPLA_2$) in the anterior (AM) and posterior (PM) midgut of *R. prolixus*. Adult females were fed with blood containing 10^8 parasites/ mL and 3, 10, 15 and 21 days after feeding insects were dissected. AM and PM were collected and submitted to total RNA extraction, cDNA synthesis and real time PCR for the analysis of four $sPLA_2$, $RpPLA_2$ 4037, $RpPLA_2$ 9995, $RpPLA_2$ 8617 and $RpPLA_2$ 8619 gene expression. It was observed that, in the AM, the expression of $RpPLA_2$ 4037 was significantly reduced 21 days after feeding and $RpPLA_2$ 8617 gene expression significantly increased 10 days after feeding. In the PM, a significant reduction in the expression of $RpPLA_2$ 4037, $RpPLA_2$ 8617 and $RpPLA_2$ 8619 on the third day after infection was observed. Furthermore, the expression of $RpPLA_2$ 4037 reduced fifteen days after infection, $RpPLA_2$ 9995 reduced ten and fifteen days after and $RpPLA_2$ 8617 increased 21 days after infection. These results demonstrate that *T. cruzi* can modulate the gene expression of $sPLA_2$ in the midgut of the vector and more studies are necessary to evaluate the importance of some of those genes for parasite's proliferation and differentiation. **Supported by:**CAPES, CNPq, FAPERJ **Keywords:**Rhodnius prolixus;Trypanosoma cruzi;Phospholipase A2.

PV-34 - **The role of SIRT1 in *Rhodnius prolixus* lipid metabolism during *Trypanosoma cruzi* infection**

AMORIM, J.B.D.V.[1]; DE ARAUJO, M.F.C.[2]; ATELLA, G.[1].
1. UFRJ, RIO DE JANEIRO - RJ - BRA; 2. UFRJ, DUQUE DE CAXIAS - RJ - BRA.
E-mail: juliabenjamin97@gmail.com

Rhodnius prolixus is a mandatory hematophagous triatomine found in tropical regions such as Central and South America. It is one of the main vectors of Chagas disease caused by the protozoan *Trypanosoma cruzi*. After a blood meal on an infected vertebrate host, the insect ingests trypomastigotes, and the whole development of the parasite in the insect is limited to the intestinal environment. *R. prolixus* metabolism during *T. cruzi* infection is poorly understood, and classic energetic molecular sensors have not yet been described in this model. Sirtuins are a family of histone deacylases dependent on the availability of NAD+ regulating different metabolic pathways. In mammals, 7 sirtuins (SIRT1-7) have been described with different cell localization. For the first time, we described 4 sirtuins in the genome of *R. prolixus*, which were classified as RpSIRT1, RpSIRT5, RpSIRT6, and RpSIRT7 through phylogenetic analysis (unpublished). Furthermore, qPCR analysis showed that *T. cruzi* infection increased the expression of RpSIRT1 and RpSIRT7 in the hindgut. In the fat body, an increase in the expression of RpSIRT1 and RpSIRT5 was observed in males infected with *T. cruzi*. According to these results, only RpSIRT1 showed higher expression in the fat body and hindgut in insects infected with the parasite. From now on, we decided to analyze the impact of RpSIRT1 expression on the lipid metabolism of *R. prolixus* infected with *T. cruzi*. For this analysis, a dsRNA will be used to knockdown RpSIRT1 expression in *R. prolixus* females. The insects will be divided into two groups: infected with *T. cruzi* and uninfected. The insects will be dissected into the hindgut and fat body, which will be used for cDNA synthesis. Afterward, a qPCR analysis will be performed using key lipid metabolic enzymes to understand the impact of RpSIRT1 knockdown. Our project seeks to continue exploring modulators of lipid metabolism to contribute to enabling new targets for vector control. **Supported by:**CNPq, CAPES e FAPERJ **Keywords:**Rhodnius prolixus;Trypanosoma cruzi;Sirtuins.

PV-35 - **The impact of multi-mapping reads on transcriptomic and epigenomic datasets in *Trypanosoma cruzi*.**

DE LIMA, P.L.C.[1]; BELLINI, N.K.[2]; ROSÓN, J.N.[1]; BORGES, A.R.[2]; DA SILVA, M.S.[2]; PIRES, D.D.S.[2]; LIMA, A.R.J.[2]; SABBAGA, M.C.Q.B.E.[2]; DA CUNHA, J.P.C.[2].
1. UNIFESP & INSTITUTO BUTANTAN, SP - BRA; 2. INSTITUTO BUTANTAN, SP - BRA.
E-mail: pedrounb14@gmail.com

Trypanosomatids have a genomic structure organized in polycistronic regions (PTU). Specific promoters for each gene have not been described so far, thus post-transcriptional mechanisms are mainly responsible for gene expression regulation. Nascent transcript analysis could elucidate the real impact of transcription regulation on the expression levels of different coding DNA sequences (CDS), PTUs, as well as RNA classes in *T. cruzi*. We obtained nascent transcripts in a Global Run-on (GRO) assay by Br-UTP incorporation in epimastigotes (EPI). GRO-seq were sequenced in the Illumina NextSeq platform, and reads were processed and mapped to the *T. cruzi* Dm28c genome masking the rRNAs and removing reads with low-quality scores (MAPQ10) leading to an underestimation of certain gene sets due to an overrepresentation of multi-mapping reads (35% of total mapped reads). Many strategies have been described to deal with multi-mapped reads, resulting in greater gene/transcriptional quantification accuracy. The impact of the multi-reads on GRO and RNA-seq analysis was compared by considering either keeping or removing all multi-mapped reads or using algorithms, such as the Kallisto package and MMR, that use maximization expectation and read coverage based methods, respectively. We observed an important difference in the expression of transcripts from disruptive and core compartments. Now we are applying the same strategy to evaluate the impact of multi-mapping reads on epigenetic datasets, such as FAIRE-seq, and H2B.V ChIP-seq. In our initial analysis, this approach suggests a degree of correlation, however, we need to find the best mapping representation of these data sets, to obtain a real estimation of genome coverage. Future analysis will be carried out to compare transcript abundance of CDSs and PTUs, and finally, integrate them with RNA-seq, FAIRE and Chip-seq datasets to shed light on the impact of transcription regulation on the gene expression of *T. cruzi*. **Supported by:**FAPESP 18/15553-9
Keywords: Gro-seq;Trypanosoma cruzi;Multi-mapping.

PV-36 - **Genome scale metabolic models: A tool for metabolic comprehension**

MONTANARO, G.T.[1]; LIMA, A.R.J.[2]; NASCIMENTO, J.D.F.[1]; SAGOT, M.[3]; SILBER, A.M.[1].
1. INSTITUTO DE CIÊNCIAS BIOMÉDICAS, SP - BRA; 2. INSTITUTO BUTANTAN- SP - BRA; 3. INRIA - FRA.
E-mail: gabrielatmont@gmail.com

Genome-scale metabolic models (GSMM) allow for computational inspection of how metabolic networks build themselves around their constitutive reactions and metabolites. In addition, they allow for hypothesizing and for a better understanding of non-obvious metabolic connections between reactions that are directly or indirectly related. Additionally, simulations of GSMM can be experimentally validated. Aiming to understand the role of the metabolic pathways of *Trypanosoma cruzi* that are absent in *Trypanosoma brucei*, we used a *T. brucei* 927 GSMM containing 1986 reactions and 1687 metabolites as a descriptive and predictive tool of the metabolism of *T. cruzi*. As an experimental example, we developed *T. brucei* cell lines expressing the four-step histidine-glutamate pathway of *T. cruzi*, which is naturally absent in *T. brucei*. We then compared the experimental results with the predictions of adding these enzymes in the computational model of the metabolism of *T. brucei*, to determine if the insertion of a completely different pathway could disturb the metabolic flux of other reactions in the computational model. The model objective was set to biomass production with a growth rate at the lower bound of 0.077, calculated using proliferation curve data of *T. brucei* measured in our lab. The cell lines of *T. brucei* expressing the partial His degradation pathway cannot use His as a carbon source; additionally, urocanate, the metabolite generated through His degradation, diminishes cell viability during the nutritional stress assay. Using that previous information we can deploy the computational model to inspect which metabolites might be accumulating to cause this diminished cell viability. These metabolites will be later measured *in vitro*. Observing the metabolism of *T. cruzi* through a scaffold model can be one of the keys to comprehending the metabolic and evolutionary advantages this parasite may have compared to others. **Supported by:**FAPESP **Keywords:** trypanosoma;genome-scale metabolic models; metabolism.

PV-37 - **Alterations in lipid metabolism of *Rhodnius prolixus* during *Trypanosoma rangeli infection.***

NUNES, D.L.M.[1]; SOUSA, G.[2]; DE LIMA, A.C.C.[1]; ATELLA, G.[3].
1. UFRJ- RJ - BRA; 2. UNIVERSIDADE DE SÃO PAULO, - BRA; 3. UFRJ- RN - BRA.
E-mail: daienenunes7@gmail.com

Trypanosoma rangeli infects triatomine bugs, including *Rhodnius prolixus* (*Reduviidae, Triatominae*), and a variety of mammals. It is widely distributed in Central America and in the Northern part of South America. The geographical distribution of *T. rangeli* overlaps that of *T. cruzi* and the parasites share reduviid bugs as vectors. Once these parasites are transmitted by the same triatomine vectors, mixed infections may occur in both vertebrate and invertebrate hosts. *T. rangeli* has a complex life cycle, especially in the invertebrate host. After being ingested by a triatomine vector feeding on the vertebrate host, the parasite establishes an infection in the bug intestine. Proliferating epimastigotes and long trypomastigotes may penetrate the gut wall, invade the hemocoele, and produce numerous epimastigotes and trypomastigotes. In the hemolymph, parasites undergo constant division and transformation to metacyclic trypomastigotes. Subsequently, some of them invade the salivary glands from where they can be transmitted to the vertebrate host in the next feeding cycle. The objective of this work is to understand the alterations in lipid metabolism of *R. prolixus* caused by *T. rangeli infection*. *R. prolixus* were infected with *T. rangeli* (10^4 parasites/mL) and three days after infection the fat body and hemolymph were collected. The organs were subjected to lipid extraction and lipids classes were analyzed by thin-layer chromatography. We observe a decrease in the amount of triacylglycerols at the fat body of infected insects. This result was confirmed by fluorescence microscopy of the lipid droplets. In the fat body of infected insects, a smaller number of lipid droplets was observed. Our results demonstrated that *T. rangeli* was able to modulate the amount of triacylglycerol in *R. prolixus* fat body. Possibly, the parasites are modulating the lipid metabolism of their insect vector to supply their lipid demands **Supported by:**CAPES, CNPq, FAPERJ **Keywords:** Rhodnius prolixus; Trypanosoma rangeli;Lipid.

PV-38 - **Characterization of the translation initiation complex eIF3 in Leishmania infantum: identification of conserved and divergent elements within the interactome from six distinct subunits.**

DE ASSIS, L.A.; DE LIMA, G.B.; REIS, C.R.D.S.; NETO, O.P.D.M..
INSTITUTO AGGEU MAGALHÃES/FUNDAÇÃO OSWALDO CRUZ, RECIFE - PE - BRA.
E-mail: osvaldo.pompilio@fiocruz.br

The initiation of protein synthesis in eukaryotes depends on the action of the translation initiation factors, also known as eIFs, where the largest is the eIF3 complex, formed by 13 subunits in mammals (eIF3a to eIF3m). eIF3 has various and distinct roles in translation, such as binding to the 40S ribosomal subunit and facilitating its association with the mRNA, a role that requires its interaction with the eIF4F complex, bound to the mRNA 5' end. In trypanosomatids, single homologues were identified for 12 of the eIF3 subunits. Here, to better understand the diversity of eIF3 interactions in these parasites, six eIF3 subunits (EIF3A, EIF3D, EIF3E, EIF3G, EIF3I and EIF3J) were selected for expression in transgenic *Leishmania infantum* cell lines, fusioned to an HA epitope. Cytoplasmic extracts from each cell line were used to immunoprecipate each HA-tagged bait with anti-HA beads, with co-precipitated protein partners identified by mass spectrometry. Three of the baits co-precipitated with most of the other eIF3 subunits, but EIF3E, EIF3J and EIF3A showed more restricted profiles. Multiple eIF4F-like complexes are present in trypanosomatids, based on different eIF4E and eIF4G subunits, and these eIF3 subunits seems to associate differentially with them. For exemple, EIF3D and others co-precipitated with EIF4E3/EIF4G4 whereas EIF3E co-precipitates preferentially with EIF4E4/EIF4G3 and EIF3I with none. Some RNA binding proteins (such as RBP12, PUF7 and PUF10) co-precipitated with all baits, while others were found with specific subunits, such as EIF3I (RBP29 and RBP35) and EIF3J (PUF5). Several protein kinases, RNA helicases and even ribosomal proteins were also found differentially associated with the tagged baits. These results not only increase the knowledge about the interaction network for each of these eIF3 subunits, but also provide evidence that distinct eIF3 complexes, with different interacting partners might be active during translation in trypanosomatids. **Supported by:**FACEPE (APQ-1662-2.02/ 15), CNPq (401888/2013-4; 400789/2019-1; 310032/2019-9). **Keywords:**translation initiation;protein synthesis;regulation of gene expression.

PV-39 - **IDENTIFICATION OF A *Trypanosoma cruzi* UNIQUE PROTEIN KINASE GENE THAT IS ESSENTIAL FOR PARASITE DIFFERENTIATION, EDK1**

SMELTZER, P.D.A.L.[1]; GOUNDRY, A.[1]; ALVES, F.S.D.S.G.[1]; DA CUNHA-E-SILVA, N.L.[2]; LIMA, A.P.C.D.A.[1].
1. INSTITUTO DE BIOFISICA - UFRJ, RJ - BRA; 2. INSTITUTO DE BIOFISICA -UFRJ, RJ - BRA.
E-mail: priscilla.smeltzer@gmail.com

Protein kinases (PKs) are enzymes with fundamental roles in biology. In trypanosomatids there are >200 genes encoding PKs of various families, and a recent genome-wide kinome screen in *Leishmania mexicana* pointed to several PKs essential for parasite survival or for successful infection. In *Trypanosoma cruzi*, little is known about the role of PKs. From the *T. cruzi* genome, we identified 19 unique PK genes that are absent from *Leishmania* and *T. brucei*. We investigated the role of a selected putative unique PK, using CRISPR/Cas9 to generate null mutants in *T. cruzi* Dm28. Template DNA for guide RNAs targeting the 5' and 3' regions of the gene and for repair cassettes containing antibiotic-resistant genes were generated by PCR and introduced in epimastigote forms of the *T. cruzi* line constitutively expressing SpCas9 and T7 RNA polymerase. Populations resistant to both antibiotics were cloned and gene deletion in both alleles was verified. Null mutants were found to be incapable of differentiating from epimastigotes to metacyclic trypomastigotes (metacyclogenesis) *in vitro*, both spontaneously and by induction with TAU and TAU3AAG media. Discrimination between the different forms of the parasite was made by analyzing the kinetoplast position in relation to the nucleus, revealing that the population of the parental Dm28SpCas9 line had 24% of metacyclics, 33% of intermediate forms (Ia, Ib and/or Ic) and 43% of epimastigotes. In contrast, no metacyclic or Ic forms were observed for the null mutants. Based on this phenotype we named this PK, Essential for Differentiation Kinase (EDK1). To further validate its role, re-expressor lines were generated by re-introducing one copy of EDK1 in the tubulin locus of the null mutant. The complemented line differentiated to metacyclics and was resistant to lysis by human serum, indicating that it restored differentiation to fully functional trypomastigotes. This is the first description of a PK essential for metacyclogenesis in *T. cruzi*. **Supported by:**UK RESEARCH AND INNOVATION (GCRF); CNPq; FAPERJ **Keywords:**KINASE;DIFFERENTIATION;UNIQUE.

PV-40 - ***Leishmania braziliensis* RuvB-like1: the biological relevance of arginine methylation**

SILVA, M.L.; CAMPAGNARO, G.D.; LORENZON, L.B.; CRUZ, A.K..
FACULDADE DE MEDICINA DE RIBEIRÃO PRETO - USP, RIBEIRÃO PRETO - SP - BRA.
E-mail: mari.loterio@hotmail.com

RuvB-Like 1 is a protein belonging to the AAA+ family of ATPases. This protein is conserved from yeast to humans and is known to play essential roles in several cellular processes, such as DNA repair, transcription regulation, chromatin remodeling and apoptosis. Furthermore, RuvB-Like 1 proteins are characterized by the presence of conserved motifs identified as Walker A, Walker B, sensor I and sensor II, responsible for ATP binding and hydrolysis. In previous work we identified RuvB-Like 1 as a potential target of the *Leishmania braziliensis* Protein Arginine Methyltransferase 5 (*Lbr*PRMT5), which methylates arginine residues. Thereby, our goal is to characterize the RuvB-Like 1 in *L. braziliensis* (*Lbr*RuvB1) and the effect of arginine methylation on this protein. The alignment of amino acid sequences from humans, yeast, *Plasmodium falciparum* and *Leishmania spp*. showed that this protein is conserved in *Leishmania* parasites and 64,25% identical to the human enzyme. Also, *Leishmania* RuvB-Like 1 protein possesses the motifs Walker A, Walker B, sensor I and sensor II, suggesting they are functional. Despite the observed conservation, an overlapping of predicted structures of *Lbr*RuvB1 and human RuvB1 revealed some differences. To investigate the *Lbr*RuvB1, we added an N-terminal myc tag to *Lbr*RuvB1 and confirmed its integration by PCR, using CRISPR/Cas9 system. Western blotting assays revealed a protein of the predicted size (50 kDa) and showed that it is expressed in procyclic, metacyclic and, to a lesser extent, in axenic amastigote forms. In addition, transcripts for *Lbr*RuvB1 were present in all three biological forms and were elevated in amastigote. Preliminary results from immunofluorescence assays indicate that LbrRuvB1 displays a cytosolic localization. *Myc-LbrRuvB1:LbrPRMT5*-knockout mutants are being generated, which will allow us to assess the relevance of arginine methylation for the expression, stability and localization of LbrRuvB1. **Supported by:**Fapesp - 2020/14059-0 **Keywords:**RuvB-Like1;Leishmania;Protein Arginine Methyltransferase.

PV-41 - **Analysis of target transcripts identified through comparative proteomics of Lutzomyia umbratilis, the main vector of Leishmania (Viannia) guyanensis in the Amazonian region, in populations with different vectorial capacities.**

DE ALMEIDA, V.R.[1]; SPELTA, G.I.[1]; DOS SANTOS, E.F.M.[2]; PESSOA, F.A.C.[2]; VELÁSQUEZ, C.M.R.[2]; BATISTA, M.[3]; MARCHINI, F.K.[3]; TEMPONE, A.J.[1]; COELHO, F.S.[1]; TRAUB-CSEKO, Y.M.[1].
1. INSTITUTO OSWALDO CRUZ, RJ - BRA; 2. INSTITUTO LEONIDAS E MARIA DEANE, AM - BRA; 3. INSTITUTO CARLOS CHAGAS, PR - BRA.
E-mail: victorramos9@hotmail.com.br

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. We performed midgut proteomic analysis using sand flies from both cities. A total of 3,292 proteins were identified, 3,252 of which are found in sandflies from both locations, 30 are exclusively present in MNP and 11 only in RPE. Among the proteins shared in both populations, four were selected for transcriptional validation, based on the criteria of having a fold change greater than 2 or less than 0.5 and a potential to impact on the vectorial capacity of insects. Of these four proteins, SL9B2, PATH and PEPT1-Like may be correlated with the process of alkalinization of the insect's intestinal lumen, affecting the parasite development, and Paramiosin is correlated with the protective capacity of the sand fly's digestive tract muscles, which would also impact the vector-parasite interaction and thus explain the epidemiological difference observed in the two cities. 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 PATH, PEPT1-Like and Paramiosin are upregulated in MNP, similar to the data from the proteomic analysis and these being probably relevant in the mechanism of parasite transmission by the insect. On the other hand, the protein SL9B2 had more transcripts in sand flies from RPE, different from the proteomic result and, although it is known that levels of transcription are not always related to abundance of proteins, the influence of this protein in the interaction of the parasite with the insect still needs to be better analyzed. **Supported by:**FAPERJ **Keywords:**Leishmania;Sand fly;Vectorial capacity.

PV-42 - **Restoring of a pseudogene encoding a nucleoside hydrolase enzyme from the parasite *Trichomonas vaginalis***

VALÉRIO, C.D.D.[1]; DE FARIAS, K.L.[1]; ROSA, R.S.D.L.[2]; RENNÓ, M.N.[1]; NEPOMUCENO-SILVA, J.L.[1].
1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RJ - BRA; 2. INSTITUTO OSWALDO CRUZ, RJ - BRA.
E-mail: carlosdvalerio@gmail.com

In order to survive and to successfully infect the human host, the parasite *Trichomonas vaginalis* depends on purine and pyrimidine salvage pathways, and nucleoside hydrolases (NHs) are the main enzymes involved in nucleoside metabolism to obtain new nucleobases. The genome of *T. vaginalis* (G3 strain) encodes four NHs (TvNH1-4), as well as a more distant related pseudogene (TvNH5), which has its ORF interrupted by a stop-codon. We have sequenced TvNH5 from two *T. vaginalis* strains (JT and FMV1) and confirmed the same interruption in both genes. Furthermore, we found an apparently intact orthologue of TvNH5 in *Tritrichomonas foetus* genome (Belfast strain), sharing 63% similarity in aa sequence. Thus, the inactivation of TvNH5 gene seems to have occurred at the basis of *T. vaginalis* speciation. This study aims to investigate the activity of an artificially restored TvNH5, in order to understand putative selective pressures that caused its inactivation. First, we have produced a 3D homology-based model of a potentially functional TvNH5 (TvNH5-T544C), inserting a gln residue in the stop-codon position, based in the corresponding sequence from *T. foetus*. TvNH5-T544C was built using the Swiss-Model server, based on an NH structure from *Gardnerella vaginalis* (6BA0). Refinement was performed with the Galaxy WEB server and the quality of the model was availed using Verify 3D, PROCHECK and Prosa-Web servers. The nucleoside substrate specificity of TvNH5-T544C will be evaluated by molecular docking. Next, using site-directed mutagenesis, we have produced the TvNH5-T544C gene, replacing the TAA stop-codon by a CAA gln codon. The TvNH5-T544C gene was fused with the iLOV fluorescent gene and cloned in the *T. vaginalis* expression vector pMasterNeo, in order to study cellular localization and possible phenotypic effects. TvNH5-T544C was further cloned in the vector pET15b, for heterologous expression and production of a recombinant protein to test in vitro for substrate specificity.**Keywords:**Trichomonas vaginalis;nucleoside hydrolase;pseudogene.

PV-43 - **Geographical Origin of *Leishmania* Ross, 1903 (Trypanosomatidae)**

GAMA, A.J.D.S.; GONÇALVES, I.C.; LOPES, A.H.D.C.S..
UFRJ- RJ - BRA.
E-mail: annegama21@gmail.com

Several hypotheses have been proposed on the origin of *Leishmania*. The Neotropical hypothesis places its origin in the Neotropical region, with mammals as their first vertebrate host. The Palearctic hypothesis proposes an origin in the Palearctic region during Cretaceous and reptiles would be their first vertebrate hosts, dispersing via terrestrial bridges subsequently. The Supercontinent hypothesis suggests an origin in Gondwana, with mammals as their first vertebrate host and vicariant events playing a major role in the lineage distribution. The aim of this work is to discuss the geographical origin of *Leishmania*, suggesting historical processes that may have influenced current patterns of distribution of the genus based on hypotheses of phylogenetic relationships and reconstructing of ancestral areas. Gene sequences from gGAPDH, HSP70 and V7V8 were retrieved from GenBank and TriTrypDB in a total of 45 terminal taxa (29 from *Leishmania*). 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 (S-DIVA). Bayesian analysis recovered *Leishmania* and its subgenera as monophyletic with high support values. *Sauroleishmania* placement deep within the tree suggests mammals as the first vertebrate hosts of the genus. S-DIVA analysis does not support a Palearctic origin. Highest probability areas recovered were Afrotropical+Neotropical region (p=22%), or Neotropical region (p=21%) or Neotropical+Oriental region (p=21%). Recovered geographical events follow the Supercontinent hypothesis. *Viannia* lineage remained on Neotropical region while *Leishmania+Sauroleishmania* dispersed from Africa to other regions. Geographical history of *Mundinia* could not be recovered by the S-DIVA analysis. Further molecular clock analyses are needed to better understand *Leishmania* evolution. **Supported by:**CNPq
Keywords: Molecular Phylogeny;Biogeography;S-DIVA.

PV-44 - **Antileishmanial activity of metal complexes and sidnones in Leishmania amazonensis**

DE SOUZA, W.S.[1]; BARBOSA, I.R.[2]; DE ASSIS, V.A.M.[3]; MACHADO, S.D.P.[3]; ECHEVARRIA, A.[2]; DE ANDRADE-NETO, V.V.[4]; TORRES-SANTOS, E.C.[4].
1. LABORATÓRIO DE BIOQUÍMICA DE TRIPANOSOMATÍDEOS, INSTITUTO OSWALDO CRUZ, FIOCRUZ, - BRA; 2. DEPARTAMENTO DE QUÍMICA, UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO, RJ - BRA; 3. INSTITUTO DE QUÍMICA, UFRJ- RJ - BRA; 4. LABORATÓRIO DE BIOQUÍMICA DE TRIPANOSOMATÍDEOS, INSTITUTO OSWALDO CRUZ, FIOCRUZ- RJ - BRA.
E-mail: waleska.soares.16@gmail.com

Leishmaniases are classified as neglected diseases, as they are not attractive for the pharmaceutical industries, since there is no prospect of a return on financial investment. Furthermore, there is no licensed vaccine for use in humans, the current treatment is expensive, toxic, painful and many cases of resistance have been reported, making treatment difficult. With the advance in inorganic chemistry, metal complexes have been rescued as interesting alternatives for drugs. In fact, gold, platinum, and silver complexes have shown good activity against tumors and some infections. On the other hand, sidnones constitute the first class of synthesized mesoionic compounds, having important activity biology and are extensively studied. This work proposes structure/activity studies to evaluate the effect of organometallic complexes and mesoionic derivatives in *Leishmania amazonensis*, the species responsible for the cutaneous form. *L. amazonensis* promastigotes were incubated with the molecules at different concentrations for 72 hours and the parasite growth was evaluated with resazurin. To evaluate the anti-amastigote activity, murine peritoneal macrophages were infected for 4 hours, treated with molecules for 72 hours, and the activity was evaluated microscopically. For cytotoxicity assays, murine peritoneal macrophages were incubated with the molecules for 72 hours and cell viability was evaluated by resazurin. In silico pharmacokinetic and toxicological analysis was also performed for the active substances, as a selection parameter for candidates to be evaluated in vivo. Of the 15 sidnones evaluated in this work, eight showed activity, with IC_{50} below 100µM, as well as the silver complex with an IC_{50} of 34µM. These promising compounds will be next evaluated in vivo. **Supported by:**CIENTISTA DO NOSSO ESTADO - FAPERJ E-26/202.918/2018
Keywords: Mesoionic compounds;organic-metallic complexes;sidnones.

PV-45 - **Sugar nucleotide biosynthesis pathways diverge among trypanosomatids**

FIGUEREDO, B.; DE LIZ, L.V.; MAIA, G.A.; BENETTI FILHO, V.; GRISARD, E.C.; STOCO, P.H..
UNIVERSIDADE FEDERAL DE SANTA CATARINA, FLORIANÓPOLIS - SC - BRA.
E-mail: figueredobeatriz@outlook.com

Trypanosomatids have a variety of glycoconjugates that play pivotal roles on these species infectivity, virulence and survival within hosts. Based on the description of the sugar nucleotide biosynthesis (SNB) enzymes on the TriTryps genomes (*Trypanosoma brucei, Trypanosoma cruzi*, and *Leishmania major*), we have comparatively evaluated genes coding for 21 enzymes involved in SNB among 18 trypanosomatid species, and their close relative species, *Bodo saltans*. BLASTp analyzes were carried out using the TriTryps SNB sequences as query against the TritrypDB database (v57), following search for conserved domains using the CDD Websearch tool. All analyzed species contain genes required for production of UDP-GlcNAc, GDP-Man, and UDP-Galp, while *Leishmania, Endotrypanum, Porcisia, Crithidia*, and *Leptomonas* species lack the gene encoding for GDP-L-fucose-synthetase that leads for GDP-Fuc generation. Some SNB-related genes like phosphoglucomutase, involved in the biosynthesis of UDP-Glc, and the components of UDP-Galf synthesis pathway were not observed on the African trypanosomes. The last was also absent in *Angomonas deanei*, *Blechomonas ayalai*, and *Paratrypanosoma confusum*. While genes coding for UDP-Rha and UDP-Xyl biosynthesis were observed in T*. cruzi, T. rangeli, T. grayi, P. confusum*, and *B. saltans*, in *B. ayalai* only the UDP-Rha coding gene is observed. The unique known gene in the GDP-Ara biosynthesis pathway, fucose (arabinose) kinase, is absent in *P. confusum, B. saltans, A. deanei*, and all African trypanosomes. The differences observed on sugar biosynthesis pathways among trypanosomatids reflects their genome plasticity and might be related to distinct nutritional requirements or availability across a variety of hosts and vectors involved on their distinct life cycles. Thus, detailed investigation of sugar nucleotide pathways in trypanosomatids will contribute to enlighten important metabolic routes, metabolites and general glycobiology of parasites. **Supported by:**CNPq, CAPES, FINEp **Keywords:**Monosaccharides;Trypanosomatidae;Evolution.

PV-46 - ***Herpetomonas muscarum* in *Stomoxys calcitrans* (Linnaeus, 1758): isolation, identification and interaction aspects.**

ROSA, D.[1]; FLORENCIO, M.C.M.[1]; SANTOS, H.L.C.[2]; DA SILVA, L.H.P.[1]; DO NASCIMENTO, A.A.[1]; MENNA-BARRETO, R.F.S.[2]; FAMPA, P.[1].
1. UFRRJ, SEROPÉDICA - RJ - BRA; 2. FIOCRUZ, RIO DE JANEIRO - RJ - BRA.
E-mail: pfampa@ufrrj.br

Stomoxys calcitrans (Diptera) or stable fly is a cosmopolitan insect and the only species of the genus that occurs in America. Adult females and males are hematophagous, being mechanical vectors of a wide range of pathogens of veterinary interest as viruses, bacteria, protozoa and helminths. Their bites are painful and cause a lot of stress to livestock hosts affecting weight gain and causing reduced milk production. The Trypanosomatidae family includes both heteroxenic protozoa of medical and veterinary importance, as well as monoxenic representatives found only in arthropods. Monoxenic protozoa have potential as a strategy for biological control of insect vectors. Recently, we observed the presence of a trypanosomatid, molecularly characterized by our group as *Herpetomonas muscarum* in *S. calcitrans* hemolymph of field and F1 colony flies kept in our laboratory. The isolates were cultured and cryopreserved in liquid nitrogen and deposited in the Protozoa Collection of Fundação Oswaldo Cruz, RJ. In the present work, besides describing the isolation and identification, we analyzed the *in vitro* and *in vivo* interaction of *H. muscarum* with *S. calcitrans* guts. *H. muscarum* remained bound to the fly guts in higher numbers when compared to *Leishmania amazonensis* and was detected in the fly guts until three days after being ingested by the insect. Ultrastructural and histological aspects of *H. muscarum – S. calcitrans* guts are being analyzed by electron microscopy. Moreover, we observed that *S. calcitrans* hemocytes are classified as plasmatocytes, granulocytes, oenocytoids and prohemocytes and we intend perform differential quantification of these cell types in flies infected or not with the trypanosomatid. *S. calcitrans* is considered a pest of livestock and yet there are not available effective ways of controlling its proliferation and we expect to better understand its relationship with *H. muscarum* and how it affects the insect biology. **Supported by:**Capes, Faperj e PROPPG-UFRRJ **Keywords:**mechanical vector;monoxenous trypanosomatids;immune system.

PV-47 - **The evolution of FLA1 and FLA1BP proteins among Trypanosomatids**

DE LIZ, L.V.[1]; FIGUEREDO, B.[1]; D'AGOSTINI, L.S.[1]; DA SILVA, V.S.[1]; GRISARD, E.C.[1]; SUNTER, J.D.[2]; STOCO, P.H.[1].
1. UNIVERSIDADE FEDERAL DE SANTA CATARINA, SC - BRA; 2. OXFORD BROOKES UNIVERSITY, ENG
E-mail: figueredobeatriz@outlook.com

Trypanosomatid's single flagellum is attached to the cell body membrane through a specialized region called the flagellum attachment zone (FAZ). In *Trypanosoma brucei* procyclic forms, the Flagellar Adhesion Glycoprotein 1 (FLA1) and FLA1-Binding Protein (FLA1BP), are localized in the cell body and flagellar membrane, respectively, and interact to attach the flagellum to the cell body through their extracellular regions, which contain an NHL domain. FLA1 and FLA1BP have a transmembrane region and an intracellular tail, which is important for their FAZ localization. A paralogous set of FLA proteins are expressed in *T. brucei* bloodstream forms, with similar functions to FLA1/FLA1BP. However, little is known about the pattern of FLA protein conservation in other trypanosomatids. Comparative sequence analysis of the 7 FLA proteins from *T. brucei* revealed that FLA1 and FLA1BP are single copy genes in all analyzed species, except for *Trypanosoma congolense*, for which, paralogous genes are observed in two distinct chromosomes. This gene expansion may have allowed the stage-specific gene expression regulation between the different life cycle stages of *T. brucei*. The sequence of FLA1BP varies among species; however, the NHL domain within the extracellular region is conserved, likely mediating the FLA1-FLA1BP interaction. While the intracellular region of FLA1BP varies in sequence and size among species, the initial 13 residues after the transmembrane region (IR^{13}) are highly conserved. To assess the importance of this region, we overexpressed in *Trypanosoma cruzi* RFP tagged *Tc*FLA1BP and *Tc*FLABP1ΔIR^{13}. Overexpression of *Tc*FLA1BP resulted in altered parasite growth and morphology while parasites overexpressing *Tc*FLABP1ΔIR^{13} were less affected. Taken together, our results show that FLA1/FLA1BP gene family have expanded in *T. brucei* and *T. congolense*, while other trypanosomatids have a single pair of FLA1/FLA1BP. **Supported by:** CNPq, CAPES, FINEp, Oxford Brookes University **Keywords:**Trypanosomatidae ;Flagellar adhesion; Intracellular junction.

TB-12 - **Assessing the efficacy of MMV-Covid Box drugs on tachyzoites of Toxoplasma gondii**

COSTA, A.L.O.; DOS SANTOS, M.; LOPES, R.E.N.; DUARTE, E.D.S.M..
UNIVERSIDADE FEDERAL DE MINAS GERAIS, MG - BRA.
E-mail: andreialocosta@hotmail.com

Despite the medical relevance of toxoplasmosis, only a few drugs are available for its treatment. Additionally, some parasite strains are already resistant to the current chemotherapy. Besides, all available drugs only act on the acute phase of the disease and are associated with several side effects, often leading to treatment interruption. Thus, the discovery of new treatments for toxoplasmosis is imperative. In the current study, an initial in vitro screening was conducted using drugs from the initiative Medicines for Malaria Venture (MMV)'s Covid Box, which has 160 drugs that can potentially be repositioned for the treatment of neglected infectious diseases. Screening of compounds was done using 6-well plates seeded with NHDF cells in supplemented RPMI-1640 medium and maintained at 37 ºC and 5% CO2. Cells were infected with 600 tachyzoites of RH strain. Each infected well was treated with 1 µM of one of the 160 drugs for 7 days. Antiproliferative activity was assessed by analyzing plaque size using ImageJ. Out of the 160 drugs, 18 were able to inhibit tachyzoite proliferation in more than 70%. Assays to evaluate the cytotoxicity and IC50 of the best drugs in inhibiting tachyzoite proliferation are being performed. **Supported by:**CNPq, CAPES, FAPEMIG. **Keywords:**Toxoplasmosis;drug and compounds repositioning;treatment.

TB-13- **Unravelling the resistance mechanisms of paromomycin in *Leishmania amazonensis***

COSER, E.M.[1]; VERGARA, P.T.[2]; GREIF, G.[3]; SANTOS, Y.[4]; FERREIRA, B.A.[1]; DA SILVA, C.S.[1]; COBB, S.[4]; ROBELLO, C.A.[3]; ALVES, J.M.P.[2]; COELHO, A.C.[1].
1. UNICAMP - SP - BRA; 2. USP, SP - BRA; 3. INSTITUT PASTEUR DE MONTEVIDEO, URU; 4. DURHAM UNIVERSITY, GBR
E-mail: elizabethmcoser@hotmail.com

Paromomycin (PM) is an aminoglycoside antibiotic used in the treatment of visceral leishmaniasis in Southeast Asia. Due to the limitations of the treatment and the potential of PM, our aim is to identify potential genes associated with PM susceptibility and resistance in *L. amazonensis*, using clinical isolates with differential susceptibility to PM and PM resistant lines selected *in vitro*. The selection of PM resistant lines was done through three strategies: *in vitro* mutagenesis and stepwise selection in promastigotes and amastigotes. After confirmation of the resistance phenotype through drug susceptibility assays, we performed whole genome sequencing of clinical isolates and PM resistant lines selected *in vitro*. Potential genes involved in PM resistance are being functionally validated by gene knockout and/or gene overexpression. *CDPK1*, which is involved in PM resistance in *L. infantum*, was mutated in 3 of 5 PM resistant lines selected by *in vitro* mutagenesis, but not in a clinical isolate that is intrinsically resistant to PM. We inactivated *CDPK1* gene by CRISPR/Cas9 and confirmed its role in PM resistance in *L. amazonensis. L23a* is a ribosomal protein involved in translation that interacts with CDPK1 and was previously involved in PM and antimony resistance in *Leishmania* spp. We generated knockout lines for this gene in *L. amazonensis* and the transgenic lines were resistant only in the promastigote form. Moreover, the PM accumulation in isolates with differential susceptibility was evaluated by fluorescence microscopy and flow cytometry, using a fluorescent analog of PM. We found a direct correlation between PM susceptibility and accumulation of this drug in this species, indicating that a transporter may be involved in the resistance phenotype. This study will contribute for the identification of genes involved in resistance and susceptibility to PM in *Leishmania* that can be potentially useful as markers of resistance in endemic areas where PM is used. **Supported by:**FAPESP (Processo 2019/22175-3) **Keywords:**Paromomycin;Drug Resistance;Whole Genome Sequencing.

TB-14 - **Evaluation of in vivo susceptibility of atypical strains of *Toxoplasma gondii* with a profile of resistance to SDZ and PYR to the alternative drugs used for the treatment of toxoplasmosis**

VIEIRA, G.C.D.; COSTA, A.L.O.; DOS SANTOS, M.; VITOR, R.W.D.A.; MARTINS-DUARTE, É.D.S..
UNIVERSIDADE FEDERAL DE MINAS GERAIS, MG - BRA.
E-mail: giuliacdv@outlook.com

Toxoplasma gondii is an obligate intracellular protozoan responsible for causing toxoplasmosis. In Brazil, toxoplasmosis has a seroprevalence ranging from 40 to 80% among adult individuals. Currently, the gold-standard treatment for toxoplasmosis consists of the combination of sulfadiazine (SDZ) and pyrimethamine (PYR), and alternative drugs such as sulfamethoxazole (SMX), trimethoprim (TMP), clindamycin (CLN), and atovaquone (ATV) may also be used. There are reports of treatment failures due to parasite resistance, especially in Brazil. Genetic diversity among strains from South America may be the reason for the differences in the pattern of susceptibility to treatment. Previous studies by part of this group reported four atypical strains of *T. gondii* with decreased susceptibility to SDZ and PYR. Therefore, the objective of this study was to study the in vivo susceptibility of these atypical strains (CTBr4, CTBr11, CTBr17, and CTBr23) against the alternative drugs used for the treatment of toxoplasmosis. In vivo assays were performed using female Swiss mice i.p.-infected with 10000 tachyzoites of the four different strains. Mice were then distributed in groups administrated for 10 days with different dosages and associations of SDZ, PYR, SMX, TMP, CLN, and ATV. Mice mortality was monitored daily for 42-days. Survival curve analysis showed that treatment with SDZ and PYR showed good responses only in CTBr11. Different dosages of SMX and its association with TMP tended to increase the survival rate in all studied strains. CLN treatment led to a mice survival rate higher than 80% in CTBr11 and CTBr23 groups but a high rate of failure was seen in mice infected with CTBr4 and CTBr17. No survival differences were seen among groups treated with ATV. Some strains showed resistance characteristics to the drugs studied as in the ATV, SDZ, PYR and CLN groups. Additional assays can be performed to better characterize susceptibility in vitro. **Supported by:**Conselho Nacional de Pesquisa (CNPq); Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). **Keywords:**Treatment of toxoplasmosis;Resistance;Alternative drugs.

TB-15 - **Genomic characterization and functional analysis of miltefosine transporter gene of *Leishmania braziliensis***

DA SILVA, C.S.[1]; COSER, E.M.[1]; VERGARA, P.T.[2]; GREIF, G.[3]; ROBELLO, C.A.[3]; ALVES, J.M.P.[2]; COELHO, A.C.[1].
1. UNICAMP, - SP - BRA; 2. USP, SP - BRA; 3. INSTITUT PASTEUR DE URU
E-mail: crissaborito@gmail.com

Cutaneous leishmaniasis in Brazil is caused mainly by *Leishmania braziliensis*. There are no vaccines for leishmaniasis and the treatment has a limited number of drugs that are toxic and induce several side effects. Miltefosine is an alternative drug that was recently approved for the treatment of cutaneous leishmaniasis in Brazil. Recent studies have shown a significant variation in *in vitro* susceptibility to miltefosine in *L. braziliensis* isolates that were never exposed to miltefosine, suggesting that intrinsic resistance may occur in this species. The uptake of miltefosine in *Leishmania* is mainly performed by the miltefosine transporter (MT) in association with its subunit Ros3. In this study, our aim is to understand the role of MT in miltefosine susceptibility in *L. braziliensis* through strategies like overexpression and knockout by CRISPR/Cas9 technology. We also performed whole genome sequencing of *L. braziliensis* (M2903 strain) and our previous analysis indicated that there are three copies of *MT* gene, differently for what is reported at TriTrypDB, where only two copies are annotated. Sequence analyses of these three copies indicated some SNPs, but it still is unknown if these changes may affect miltefosine susceptibility in this species. The episomal overexpression of one of these *MT* gene copies in a heterologous species, *L. amazonensis*, indicated that this gene is functional and transgenic parasites were more susceptible to miltefosine than parasites transfected with the empty vector. Furthermore, we have already generated a *L. braziliensis* line with at least two copies of *MT* gene inactivated by CRISPR/Cas9. Miltefosine susceptibility assays with these transgenic lines is in progress. Our findings will be helpful to predict whether MT may also affect the effectiveness of this drug against this species, and whether treatment with alternative drugs is need when MT is not functional, for example. **Supported by:**FAPESP (Processo 2021/00171-6) **Keywords:** Leishmania braziliensis;Miltefosine;Miltefosine Transporter.

TB-16 - **A methodology to search specific protein synthesis inhibitors for *Trypanosoma cruzi***

MANTOVANI, C.C.[1]; AKTAS, B.H.[2]; SCHENKMAN, S.[1].
1. UNIVERSIDADE FEDERAL DE SÃO PAULO, - SP - BRA; 2. BRIGHAM WOMEN'S HOSPITAL, USA.
E-mail: huseyin_aktas@hms.harvard.edu

Chagas disease (CD) is a public health issue and due to emigration of infected people to non-endemic, CD has been affecting the global population. It is estimated that over 6 to 7 million people worldwide are infected and about 75 million are at risk of contamination. The disease is caused by *Trypanosoma cruzi*, and its protein synthesis is distinct from other eukaryotic organisms because of significant differences in their ribosomes. Blocking protein synthesis is the mechanism of many employed antimicrobials nowadays. Therefore, our main goal in this project was to establish a *T. cruzi* competent cellular extract assay that can translate a messenger RNA codifying the luciferase enzymes, which can be detected by light production as the purpose of finding new inhibitors to be used as therapeutic treatments for CD. In this work we obtained translation competent extracts from cultured epimastigotes from a non-virulent clone derived from DM28c strain. The extracts were prepared by nitrogen cavitation lysis followed by centrifugation and stored frozen at -80 C. The extracts were able to translate capped and polyadenylated RNA synthesized in vitro. The resulting mRNA containing either Renilla or Firefly luciferase and a 3' UTR region of tubulin was incubated with the cell extract in a multiwell format and generated luminescence that was abolished by protein synthesis inhibitors. This assay is therefore useful to select compounds able to inhibit specifically *T. cruzi* protein synthesis that might be used in treatment of Chagas disease. **Supported by:**CNPq-Pibic
Keywords: Trypanosoma cruzi;Translation;Protein synthesis inhibitors.

TB-17 - **CHARACTERIZATION OF TRYPANOSOMA CRUZI eIF2α PROTEIN KINASE 3**

YAMAGUCHI, P.; MARCELINO, T.D.P.; SCHENKMAN, S.
. UNIFESP, SP - BRA.
E-mail: pri_yamaguchi@yahoo.com.br

Trypanosoma cruzi, the protozoan that causes Chagas disease, is exposed during its life cycle to changes in temperature, pH, nutrients, osmotic or ionic variations and to oxidizing agents. Thus, these organisms need to adapt to different environments, controlling changes in their metabolism, followed by changes in gene expression and morphology. Adaptive stress responses are in part regulated by protein kinases that phosphorylate translation initiation factor 2 (eIF2) inhibiting general protein synthesis and promoting the expression of stress responsive genes. One of the candidate protein kinases predicted to phosphorylate eIF2 in *T. cruzi* is called K3, based on its sequence similarity to eIF2 protein kinases in other organisms. To characterize the enzyme, we cloned and expressed its kinase domain in *E. coli.* We obtained a recombinant protein that generates ADP as detected by ADP-Glo assay, suggesting it could be autophosphorylated, but it could not phosphorylate Creb, p70-S6, or histone H3, while the first two substrates were phosphorylated by other *T. cruzi* eIF2 kinases. We also generated specific antibodies by immunizing rabbits with the recombinant protein and generated a mNeonGreen-Myc tag in the parasite employing Crispr/Cas9. Experiments to identify the enzyme substrates in vitro and to localize the protein in normal conditions or upon stress are underway.
Keywords: Trypanosoma cruzi;Kinase 3;eIF2.

TB-18 - **Complete assembly, annotation of virulence genes and CRISPR editing of the genome of *Leishmania amazonensis* PH8 strain**

GOES, W.M.; BRA, C.R.F.; CUNHA, J.L.R.; COQUEIRO-DOS-SANTOS, A.; SOUTO, T.C.; FERNANDES, A.P.S.M.; LARANJEIRA-SILVA, M.F.; BARTHOLOMEU, D.C.; TEIXEIRA, S.M.R..
UNIVERSIDADE FEDERAL DE MINAS GERAIS, MG - BRA.
E-mail: wanessa_goes@hotmail.com

Remoto Poster Session

Leishmania amazonensis is one of the etiological agents of cutaneous leishmaniasis. Genome studies focused on parasite genes encoding virulence factors that play crucial roles in the establishment of the infection constitute an essential step towards the molecular characterization of a protozoan parasite that is being increasingly recognized as a significant human pathogen. Here, we report the sequencing and assembly of the *L. amazonensis* PH8-strain combining data from long PacBio reads, short Illumina reads and synteny with the *Leishmania mexicana* genome. The final assembly, composed of 34 chromosomes, represents a genome of ~ 32 Mb with 8225 annotated genes. Several multigene families encoding virulence factors, such as A2, amastins, metalloproteins GP63 and cysteine proteases, were identified and compared to their annotation in the genome of other Leishmania species. The *L. amazonensis* PH8-strain genome has 27 genes encoding all four sub-classes of amastins (α, β, γ and δ), 5 genes genes encoding A2 antigens, 8 genes encoding the metalloprotease GP63 and 48 genes encoding cysteine proteases. As they have been recently recognized as virulence factors essential for disease establishment and progression of the infection, we have also identified 13 genes encoding proteins involved in the parasite iron and heme metabolism and compared to this gene repertoire in other Trypanosomatids. To follow these studies with a genetic approach that would allow to directly address the role of these virulence factors, we tested two CRISPR-Cas9 protocols to generate *L. amazonensis* knockout cell lines. Using the Miltefosine Transporter gene as a proof of concept, we transfected promastigotes expressing the *Streptococcus pyogenes* Cas9 with in vitro transcribed sgRNA targeting this gene. Alternatively, promastigotes were transfected with recombinant *Staphylococcus aureus* Cas9 complexed with sgRNAs. With both strategies we were able to disrupt the TM gene with high efficiency. **Supported by:**CAPES, INCTV
Keywords: Leishmania amazonensis;virulence factors;CRISPR editing.

TB-19 - **P2X7 receptor participating in intestinal infection control in mice infected with *T. gondii* – EGS strain**

RANGEL, T.P.; MOREIRA-SOUZA, A.C.D.A.; SILVA, S.R.B.; VOMMARO, R.C.; SILVA, R.C..
UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RJ - BRA.
E-mail: vommaro@biof.ufrj.br

The classic immune response against *Toxoplasma* involves secretion of IL-12 by innate immune cells, with the consequent activation of other innate or adaptive immune cells that secrete IFN-γ, which activated microbicidal mechanisms in infected cells (YAROVINSKY, 2014). The P2X7 receptor (P2X7R) belongs to the purinergic receptor family, with ATP as its activator molecule. P2X7R activation has been shown to be an important mechanism of control of *T. gondii* infection. 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 (MOREIRA-SOUZA AND COUTINHO-SILVA R., 2021) in the context of Toxoplasma infection. In this work, we investigated the P2X7 receptor contribution during intestinal inflammation induced by the EGS strain of *T. gondii.* The EGS strain was isolated in 1998 from the aminiotic fluid of a patient in Minas Gerais – Brazil and is a recombinant genotype (I/III) (VIDIGAL et al., 2002; FERREIRA et al., 2004). C57black/6 wild-type mice (WT), and P2X7R knockout ($P2X7^{-/-}$) mice were analyzed after 8 days post-infection with the EGS strain. The infection induced an increase in morbidity in all infected animals. When we observed the small intestine of infected animals, we found a reduction in tissue length, indicative of inflammation. The RT-qPCR assay showed reduction in expression of IL-12, and an increase in IFN-γ expression in $P2X7^{-/-}$ mice in comparison with WT. The parasite load was higher in $P2X7^{-/-}$ mice than in WT, when analyzed by the expression of the *Toxoplasma* B1 gene by qPCR. We conclude that the presence of P2X7 receptor is important in the controlling of parasite load, contributing to the classic immune response against *T. gondii*, during EGS strain infection. **Supported by:**CNPq, CAPES e FAPERJ **Keywords:**Purinergic signaling;Immune response;Toxoplasma Brazilian strain.

TB-20 - **Effect of histone deacetylase inhibitors as an alternative for the treatment of ocular toxoplasmosis**

SILVA, C.A.D.A.E.; DE ARAUJO, M.R.P.; DE SOUZA, W.; VOMMARO, R.C..
UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RJ - BRA.
E-mail: milenapeclat4@gmail.com

Toxoplasmosis is a cosmopolitan zoonosis caused by the obligate intracellular protozoan *Toxoplasma gondii*. Ocular toxoplasmosis can be acquired post-birth or by congenital infection. The main symptoms include coriorretinitis with loss of visual acuity or blindness. In Brazil, there is a high seroprevalence of toxoplasmosis, which varies according to the region, with a high frequency of ocular toxoplasmosis related to the presence of atypical genotype strains. The treatment of toxoplasmosis is restricted to antifolates, which present several adverse effects. The search for new alternatives is necessary to increase the therapeutic arsenal. In recent study of our group, histone deacetylase inhibitor Tubastatin A (TST) was pointed out as potential anti-*Toxoplasma* chemotherapy. In this work, we evaluated the *in vivo* effect of TST, via intravitreal administration, in an acute ocular toxoplasmosis model. Males C57Black/6 of 8-12 weeks-old were inoculated with 10^4 tachyzoites of ME49 strain, intraperitoneally. The mice were treated with a single dose of 20µg/2µl in both eyes on the tenth day of infection. The eyes were dissected and processed after 72 and 120h of treatment. Morphological and ultrascructural analysis of the retina was carried out by optical microscopy and transmission electron microscopy, respectively. The untreated group showed a large displacement of retinal pigment cells of the sensory layer, suggesting photoreceptors degeneration and swelling of choroid due to vessels dilation. In treated groups we observed preservation of retinal layers organization and choroid. TST treatment led to preservation of the retinal structure and likely the interruption of inflammatory process. These results point out histone deacetylase as targets to be explored for the development of new therapeutical protocol for local treatment of posterior uveitis caused by *T. gondii* and encourage further investigation. **Supported by:**CNPq, CAPES e FAPERJ **Keywords:**in vivo tests;ocular lesion ;intravitreal treatment.

TB-21 - **PARASITE DETECTION IN VISCERAL LEISHMANIASIS SAMPLES BY A SYBR GREEN-BASED REAL-TIME PCR USING NEW GENOMIC DNA TARGETS OF TRYPANOSOMATIDS**

TAKAMIYA, N.T.[1]; ROGERIO, L.[1]; LEONEL, J.A.[2]; VIOTI, G.[3]; OLIVEIRA, T.M.F.[3]; VALERIANO, K.C.[4]; SANTOS, I.K.F.M.[4]; PORCINO, G.N.[4]; COSTA, C.H.N.[5]; FERREIRA, T.D.S.[6]; MACHADO, T.D.O.[7]; DE ANDRADE, A.J.[8]; GURGEL-GONÇALVES, R.[7]; DE ALMEIDA, R.P.[9]; MARUYAMA, S.R.C.[1].
1. UNIVERSIDADE FEDERAL DE SÃO CARLOS, SP - BRA; 2. UNIVERSIDADE, PIRASSNUNGA - SP - BRA; 3. UNIVERSIDADE DE SÃO PAULO, SP - BRA; 4. UNIVERSIDADE DE SÃO PAULO, RP - SP - BRA; 5. NATAN PORTELA INSTITUTE OF TROPICAL DISEASES, - PI - BRA; 6. UNIVERSIDAD DE BRASÍLIA, DF - BRA; 7. UNIVERSIDADE DE BRASÍLIA, DF - BRA; 8. UNIVERSIDADE FEDERAL DO PARANÁ, PR - BRA; 9. UNIVERSIDADE FEDERAL DE SERGIPE, SE - BRA.
E-mail: nayoretk@gmail.com

In Brazil, Visceral Leishmaniasis (VL) is caused by *Leishmania infantum* parasites transmitted by sandflies. Previously, we identified a non-*Leishmania* parasite isolated from an atypical and fatal case of VL. Phylogenomics of two clinical isolates revealed that both are more closely related to the monoxenous *Crithidia fasciculata* (called *Crithidia-like*). Thus, our objective was to test two new species-specific parasite genes (LinJ31_X for *L. infantum* and LVH60_12060 for *Crithidia-like*) in a SYBR Green qPCR based-assay to evaluate their potential for species identification and for quantifying parasite load in biological and experimental samples. Samples from patients and domestic animals diagnosed with VL, as well as samples from experimental infection in hamsters and in vitro infections using THP-1 cell line were used for genomic DNA isolation. DNA samples from other *Leishmania* species and *C. fasciculata* were also used as control reactions. In this work, we demonstrate that by means of calculations based on the genome of these parasites, it is possible to quantify the parasite load and detect traces of infections caused by *L. infantum* in clinical, animal, vector and experimental samples with the primer LinJ31_X. The LVH60_12060 target amplified for *Crithidia-like* and also for *C. fasciculata*, however, the specificity of the material with this target was observed through the differences in the melting curve temperature, indicating the distinction between them. In the standard curve the points varied between 1 equivalent number of parasite DNA/reaction with Cq 37.2±0.2 to 10e5 equivalent number/reaction of parasite DNA (Cq 18.5±0.1). There was the detection of parasite DNA in biological samples representing 1 equivalent number of parasite DNA (Cq 34.34± 1.8). Therefore, the LinJ31_X and LVH60_12060 primers will help in molecular diagnosis, as it was possible to determine the sensitivity in different types of samples, can be used for further investigation and screening. **Supported by:**Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP JP 2016/20258-0, and grant 2021/12464-8); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq scholarship 133661/2020-2) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES Finance Code 001). **Keywords:**Molecular Diagnosis;Leishmania infantum;Crithidia-like.

TB-22 - **Novel histone deacetylase inhibitors as an alternative for the treatment of toxoplasmosis- *in vitro* analysis**

SILVA, C.A.D.A.E.[1]; MARTINS-DUARTE, É.D.S.[2]; DE SOUZA, W.[1]; VOMMARO, R.C.[1]. 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RJ - BRA; 2. UNIVERSIDADE FEDERAL DE MINAS GERAIS, MG - BRA.
E-mail: vommaro@biof.ufrj.br

Toxoplasmosis is a cosmopolitan zoonosis, caused by the obligate intracellular protozoan *Toxoplasma gondii*. *T. gondii* infection can cause uveitis, congenital sequels, including chorioretinitis and hydrocephalus, and encephalitis in immunocompromised individuals. The treatment of toxoplasmosis is restricted to antifolate administration and has several side effects. The search for new alternatives and the understanding of the *T. gondii* biology is necessary to expand the therapeutic arsenal. In recent study of our group, histone deacetylase inhibitors, as Tubastatin A and Suberoylanilide Hydroxamic Acid were pointed out as potential anti-parasitic chemotherapy. In this work we evaluated the *in vitro* effects of novel synthesized compounds, selective inhibitors for HDAC6 derivatives of Tubastatin A (TST). LLC-MK_2 monolayers infected with *T. gondii* RH strain tachyzoites were treated with the compounds 1a, KV30 and KV24 for 48h. All the three compounds showed IC_{50} in the nanomolar range: 1a (330 nM), KV30 (320nM) and KV24 (230nM). Cytotoxicity analysis performed by the MTS essay against LLC-MK_2 showed 1a, KV30 and KV24 have high selectivity for *T. gondii*. Immunofluorescence using RH-ACP-YFP (green apicoplast) tachyzoites, α-αtubulin, α-IMC-1 (for the parasite inner membrane complex), α-Centrin-1, α-SAG-1(parasite surface antigen) and Mitotracker showed treatment of the cells with 1μM of any of the 3 compounds, for 24h, impaired parasite endodyogeny. The appearance of masses of non-individualized parasites and compromised localization of organelles, as apicoplast and the centrosomes were observed. The effects of the compounds analyzed by transmission electron microscopy confirmed the irreversible damage in the formation of new daughter cells, in parasites treated for 48h. These results corroborate HDACs as pharmacological targets for the development of new parasitic chemotherapy and indicate these compounds as potential drugs for in vivo tests with *T. gondii.* **Supported by:**CNPq, CAPES e FAPERJ **Keywords:**Chemotherapy;Histone deacetylase inhibitors;Endodyogeny.

TB-23 - **Evaluation of the leishmanicidal activity of the drug combination ezetimibe and posaconazole in *Leishmania infantum.***

DE ASSUNÇÃO, L.A.; DE ANDRADE-NETO, V.V.; TORRES-SANTOS, E.C..
FIOCRUZ, RIO DE JANEIRO - RJ - BRA.
E-mail: luizaasth8@gmail.com

Leishmaniases are part of the group of neglected tropical diseases. They are considered a series of parasitic diseases caused by different species of protozoa of the genus *Leishmania*. The treatment is based on pentavalent antimonials, miltefosine (MT), amphotericin B, and pentamidine. This group of drugs has limitations, such as high cost, toxicity, difficult route of administration, and resistance. Therefore, there is a need to develop new therapeutic strategies for the treatment of leishmaniasis. Drug repositioning is a strategy that can be used to search for more effective drugs for treatment. This work evaluated the efficacy of the combination of posaconazole (POSA) and ezetimibe (EZE) against intracellular amastigotes of *Leishmania infantum*. Miltefosine (MT) was used as a reference drug. Cytotoxicity assays of the drugs (POSA and EZE) on macrophage cultures revealed that the compounds alone and in combination and MT are not toxic to uninfected peritoneal macrophages at the concentrations tested. Intracellular amastigotes were incubated with different concentrations of the drugs alone or in combination for 72 hours. The IC_{50} in intracellular amastigotes of *L. infantum* were (POSA) 0.79 ± 1.0 μM and (EZE) 9.5 ± 1.04 μM and (MT) 0.5 ± 0.4. Finally, the combinations (POSA and EZE) at the proportions 3:2 and 1:4 in intracellular amastigotes of *L. infantum* showed a synergistic effect, indicating a promising alternative for treating visceral leishmaniasis. **Supported by:**FAPERJ E-26/200.028/2021 **Keywords:**Leishmania infantum ;Ezetimibe;Posaconazole .

TB-24 - **Evaluation of almitrine for inhibition of *Toxoplasma gondii* growth *in silico* and *in vitro***

TOLEDO, P.P.M.[1]; DE SOUSA, N.F.[2]; SCOTTI, M.T.[2]; REIMAO, J.Q.[1]. 1. FACULDADE DE MEDICINA DE JUNDIAÍ, JUNDIAÍ - SP - BRA; 2. UNIVERSIDADE FEDERAL DA PARAÍBA, JOÃO PESSOA - SP - BRA.
E-mail: juliana_reimao@yahoo.com.br

Toxoplasma gondii is an obligate intracellular protozoan parasite that belongs to Apicomplexa Phylum and is the etiological agent of toxoplasmosis. The parasite diverged from closer species due to its capability to infect a wide range of hosts, re-enforced by flexible transmission pathways. Despite the importance of toxoplasmosis to public health, considering its high prevalence in the human population and the severe clinical manifestations mainly in immunocompromised patients and in cases of congenital infection, there are still few available therapeutic options, which are effective only against the acute form of the disease. Aiming to find new uses for already known compounds, the international organization Medicines for Malaria Venture (MMV) created the COVID Box, a collection of 160 structurally diverse active compounds, set for trial against infectious diseases. Amongst them, we selected almitrine (MMV1804175) based on a previous virtual screening that indicated the existence of a *T. gondii* protein (Sodium/potassium-transporting ATPase alpha-1 chain) as its possible target. Therefore, the goal of this work was to evaluate the anti-*T. gondii* activity of amlitrine in a cell-based assay. We used a 96-well plate assays based on b-galactosidase expression to estimate the viability of *T. gondii* tachyzoites (RH strain). The cytotoxicity against mammalian cells was evaluated using human foreskin fibroblasts (HFF) cultures through the MTT assay. Almitrine presented a Half Effective Concentration (EC_{50}) value of 0.424 µM against the parasite and a Half Cytotoxic Concentration (CC_{50}) value higher than 20 µM, the top concentration evaluated. The ratio between the CC_{50} against HFF and the EC_{50} against the parasite resulted in a selectivity index greater than 47. Almitrine showed interactions with the Na+/K+ ATPase transporter for *Homo sapiens* and *Mus musculus*, indicating a possible mechanism of action of this compound. **Supported by:**FAPESP (grant number 2018/18954-4 and 2020/03399-5). **Keywords:**Toxoplasmosis;drug repurposing;drug discovery.

TB-25 - **Anti-*Toxoplasma gondii* activity of the antitumor compound milciclib and its interference in the integrity of the plasma membrane of the parasite**

REIMÃO, J.Q.; REIMAO, J.Q..
FACULDADE DE MEDICINA DE JUNDIAÍ, SP - BRA.
E-mail: juliana_reimao@yahoo.com.br

Toxoplasmosis, one of the most important parasitic diseases worldwide, is caused by the protozoan *Toxoplasma gondii* and affects approximately 25 to 30% of the world population. New and improved medicines are greatly needed to cure *Toxoplasma* infection, as no medicine eliminates the chronic, encysted form of the parasite. Milciclib is a heterocyclic compound that is under investigation in patients with malignant Thymoma. This compound is part of the “Pathogen Box” collection provided by the “Medicine for Malaria Venture” foundation. The present work aims to characterize the anti-*T. gondii* activity of milciclib and its cytotoxicity against mammalian cells, and to investigate its possible mechanism of action against *T. gondii*. The values of Half Effective Concentration (EC_{50}), Half Cytotoxic Concentration (CC_{50}) and Selectivity Index (SI) of milciclib were determined, using the drug pyrimethamine as positive control. Assays to evaluate the antiparasitic activity of milciclib were performed using *T. gondii* tachyzoites (RH strain) encoding a transgenic copy of β-galactosidase, which were maintained in confluent monolayers of human foreskin fibroblasts (HFF). Cytotoxicity assays were performed using HFF monolayers and the SI was given by the ratio between EC_{50} and CC_{50}. The integrity of the plasma membrane of milciclib-treated parasites was evaluated through the incorporation of propidium iodide, using 1% triton as positive control. Milciclib presented EC_{50} and CC_{50} values of 0.06 and 7.64 µM, respectively, resulting in an SI of 127. The compound induced immediate damage to the plasma membrane of the parasite in a dose-dependent manner. The damage to the plasma membrane of the parasite induced by milciclib may be related to cell death by necrosis. The evaluation of the efficacy of milciclib in *T. gondii*-infected animals is promising, considering its selective activity against the parasite and its good oral availability. **Supported by:**FAPESP (grant number 2018/18954-4 and 2020/03399-5). **Keywords:**Toxoplasmosis;drug repurposing;drug discovery.

TB-26 - **Establishment and validation of a medium-throughput screening method to discover new hits and leads for the treatment of leishmaniasis**

PINTO, L.A.D.S.; DANTAS, R.F.; SILVA JUNIOR, F.P.; TORRES-SANTOS, E.C..
FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRA.
E-mail: llais.abreu@gmail.com

Leishmaniasis is a serious public health problem, and it is necessary to identify new compounds, with different mechanisms of action, that can become drug candidates for the treatment of this disease. Several kinds of screening tests are performed to search for hits in chemical libraries, each one with pros and cons. Conventional phenotypic screening assays are expensive and labor-intensive, as they depend 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. To overcome these gaps in antiparasitic screening, this work proposes the establishment and validation of a medium-throughput screening methodology using intracellular *L. amazonensis*-GFP amastigotes. It is known that several reporter genes are useful in quantification methods for phenotypic assays. Then, an extensive revision was performed and a comparative table was created analyzing the advantages and disadvantages of each method and which could serve a small laboratory. In addition, the first assays for high content screening (HCS) analysis using promastigotes and intracellular amastigotes of GFP-transfected parasites and DAPI staining yielded good images, suggesting the feasibility of the technique. The next step is setting up the software to proceed with automatized quantification. **Supported by:**PIBITI - 161191/2021-5 - **Keywords:**Leishmania amazonensis ;High Content Screening (HCS);Green Fluorescent Protein (GFP).

TB-27 - **AMPHO-DEPOT®: SINGLE-DOSE INTRALESIONAL FORMULATION WITH AMPHOTERICIN B FOR THE TREATMENT OF CUTANEOUS LEISHMANIASIS**

SUZUKI, É.Y.; BORSODI, M.P.G.; GONDIM, F.C.; DE LIMA, V.K.A.; SOUSA-BATISTA, A.D.J.; BERGMANN, B.R..
UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RJ - BRA.
E-mail: erikayoko@biof.ufrj.br

Cutaneous leishmaniasis (CL) treatment remains unsatisfactory due to requirement of repeated parenteral injections that cause severe systemic side effects. Local intralesional injections with antimonials also demand repetition due to their high hydrophilicity and blood absorption that can also lead to reported systemic effects with an increase in the resistance to antimoniate. Amphotericin B (AmB) is the most active antileishmanial drug clinically available, but development of an effective topical formulation is challenging due to poor absorption through the skin. The aim of this work is to describe the development and therapeutic use of a novel AmB formulation based on sustained release delivery system for a single intralesional injection (Ampho-Depot®, mark deposited in 2021). For that, poly(lactide-co-glycolide acid) (PLGA) microparticles loaded with AmB were prepared by the emulsion solvent evaporation method and sterilized by gamma irradiation. AmphoDepot® was then characterized according to sterility, particle size distribution, zeta potential, scanning electron microscopy and encapsulation efficiency. The microparticles exhibited zeta potential of -25.3 mV, spherical shape, and mean diameter of 2.8 ± 0.4 µm (span=1.55). AmB entrapment efficiency was 73.5 ± 2.6%. No chemical or physical changes were produced by 25 kGy gamma irradiation that effectively prevented contaminated bacteria and yeast growth. *In vivo*, a single i.l. injection into *Leishmania amazonensis*-infected mouse ears revealed that Ampho-Depot® was much more effective in reducing lesion growth than the same dose of deoxycholate AmB formulation Anforicin®. Measurement of parasite burden on day 56 of infection revealed 86% parasite burden reduction as compared with vehicle controls. Anforicin® reduction was only 32%. Ampho-Depot® has strong potential as a new safe therapy, and is pharmaceutically ready for Phase IIb clinical trial in patients with CL. **Supported by:**Instituto Tecnológico Vale (ITV), CNPq and FAPERJ **Keywords:** CUTANEOUS LEISHMANIASIS; AMPHOTERICIN B;POLYMERIC MICROPARTICLES.

TB-28 - **Employment of computer-aided drug discovery to select natural compounds for leishmaniasis treatment by structure-based virtual screening**

INACIO FILHO, J.D.; DA FONSECA, M.S.; RODRIGUES, J.A.C.; LIMA, Y.D.S.; AMARAL, E.E.A..
INSTITUTO OSWALDO CRUZ, RJ - BRA.
E-mail: job@ioc.fiocruz.br

Leishmaniasis treatment remains a serious public health problem, and new oral and safe therapeutic alternatives are necessary. Over the years, a computational approach has been used to aid this search. Thus, the goal of this study was to select a natural compound using a structure-based virtual screening approach, and trypanothione reductase from *Leishmania infantum* (TrLi) was used as a target. NC2 demonstrated ΔG and estimated Ki values of -7.45 and -7.32 and 3.44 μM and 4.34 μM for 2JK6 and 4ADW, respectively. Additionally, NC2 interacts with residues participating in the catalytic site of the enzyme, supporting the hypothesis that this molecule acts as a competitive inhibitor of TrLi. To confirm their antileishmanial effect, L. infantum promastigotes were incubated with different concentrations of NC2 (0.98 – 500 μM) for 72 hours, and the viable cells were estimated fluorometrically by resazurin. The compound inhibited cellular proliferation in a concentration-dependent manner, reaching 98% inhibition at the highest concentration (500 μM), demonstrating an IC_{50} of 5.5 μM. Taken together, computational approaches can be an excellent ally to drug discovery, and our data suggest that NC2 is a safe compound administered by the oral route and inhibits *L. infantum* promastigotes, acting as a competitive inhibitor. **Supported by:**CAPES; CNPq; FAPERJ; IOC/FIOCRUZ **Keywords :**Computer-Aided Drug Discovery; Trypanothione reductase; Leishmaniasis.

TB-29 - **Use of computational approaches in a rational strategy to select natural compounds for leishmaniasis treatment by structure-based virtual screening**

DA FONSECA, M.S.; RODRIGUES, J.A.C.; LIMA, Y.D.S.; INACIO FILHO, J.D.; AMARAL, E.E.A..
FUNDAÇÃO OSWALDO CRUZ, RJ - BRA.
E-mail: myslene_fsc@hotmail.com

The treatment for leishmaniasis still presents a serious public health problem, and it is necessary to search for compounds that are less toxic, more effective, and able to be orally administered. Thus, the aim of this work was to select natural compounds for a new chemical entity using trypanothione reductase from *Leishmania infantum* (TrLi) in a structure-based virtual screening (SBVS) approach. Libraries with 65 natural compounds were obtained, and computational approaches were used to select the most promising compounds, study their molecular mechanism of action (MMA) and, therefore, characterize their antileishmanial effects. After *in silico* analyses, 5 compounds demonstrated ADMET properties compatible with oral administration and predicative low toxicity parameters. Virtual screening showed that all of the compounds interact in the active site of the enzyme, suggesting a possible interaction between the enzyme-compound complex. However, the compound NC3 was selected for presenting lower ΔG values. The MMA study calculated ΔG and estimated Ki values of -7.88 and -8.31 and 1.68 μM and 805 nM for 2JK6 and 4ADW, respectively. Additionally, the compound interacts with residues participating in the catalytic site of the enzyme, suggesting a competitive inhibition mode. To characterize its activity, an antipromastigote assay was performed. *L. infantum* promastigotes were incubated with increasing concentrations of the compound (0.98 – 1000 μM) for 72 hours, and cell viability was quantified using resazurina. NC3 inhibited 81% of parasite proliferation (1000 μM) in a concentration-dependent manner and demonstrated an IC_{50} of 331.9 μM. Together, our data demonstrated that SBVS could be an excellent alternative to select promising compounds able to be a competitive inhibitor of TRLi, encouraging us to continue to investigate their effects in *L. infantum* and suggesting that these compounds are potential candidates for oral leishmaniasis treatment. **Supported by:**CAPES; CNPq; FAPERJ; IOC/FIOCRUZ **Keywords:**Structure-based virtual screening;Trypanothione reductase;natural products.

TB-30 - **Effect of Isatin and naphtoquinones derivatives against *L. amazonensis***

GOMES, J.S.D.C.; MANGEFESTE, E.; SOARES, D.C.; HAMERSKI, L.; SARAIVA, E.M..
UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RJ - BRA.
E-mail: jangomes93@gmail.com

Leishmaniases, neglected tropical and subtropical diseases caused by parasites of the genus Leishmania, occur in 98 countries worldwide and are the second leading cause of parasite-related death. The disease manifests itself as tegumentary and visceral forms, depending on the species and the host response. There are a few available treatment options for leishmaniasis, like pentavalent antimonials, amphotericin B and its lipid formulations, miltefosine, paromomycin, and pentamidine. However, all these drugs present adverse effects, most are administered parenterally (except for miltefosine), and the emergence of resistant strains and treatment failures have been reported. Therefore, it is essential to develop less toxic drugs, more effective, and preferably for oral administration. In this study, we evaluated the effect of compounds derived from isatin and/or naphthoquinones against Leishmania amazonensis. In previous work, we demonstrated that BrMLP and BrPLP are effective against L. amazonensis promastigotes and intracellular amastigotes and are not toxic to macrophages. Aiming to enhance their effect, we modified these molecules by replacing bromine with chlorine. Here we tested the effect of ClMLP and ClPLP, analogs of BrMLP and BrPLP, respectively. These new analogues do not show toxicity to macrophages (CC50 > 200 µM) and to neutrophils (CC50 > 100 µM) and did not induce extracellular neutrophil traps (NETs) release. Our findings reveal that ClPLP has a better effect on parasite growth than the BrPLP analog, since at 5 µM, ClPLP reduced 48% of parasite viability, while BrPLP reduced 27%. They had similar effects at higher concentrations, both being able to reduce promastigote survival by 80%. On the other hand, the ClMLP analog did not improve the leishmanicidal effect compared to its analog BrMLP. BrMLP reduced parasite growth at 5 µM, and ClMLP have no effect at this concentration. **Supported by:**CNPq, CAPES, FAPERJ **Keywords:**Leishmania;natural produtcs;naphthoquinones.

TB-31 - **Development of prodrugs bioactivated by nitroreductases of *Leishmania infantum***

MONCORVO, F.M.S.U.; DA SILVA, M.B.C.; DE ANDRADE-NETO, V.V.; CARVALHO, S.D.A.; TORRES-SANTOS, E.C..
FIOCRUZ-RJ, BRA.
E-mail: fabianamaia.santos@gmail.com

The therapeutic arsenal for leishmaniasis has become obsolete, with unacceptable side effects and increasing cases of resistance nowadays. Therefore, we aim to search for new compounds that can be used as a therapeutic alternative. Several studies have shown that n-acyl hydrazones have leishmanicidal activity. Derivatives of N-acyl hydrazones were designed and synthesized to have different functional groups, such as nitroaromatic, pyrrole, furan and others, generating the PHID series. Among the molecules of the series, PHID40 and PHID121 stand out as the most potent, with IC_{50} of 2.8 µM and 1.8 µM in *L. infantum* promastigotes. After analyzing the structural differences, it was observed that the PHID121 molecule has a nitro group in its structure. Nitroderivatives have significant potential for drug development, and the foundation that made these advances possible was the identification of nitroreductases in trypanosomatids that are not homologous to humans, offering a differential for drug candidates that would be selectively bioactivated, presenting selective toxicity to the parasite, which led us to investigate its role as a prodrug and activation mechanism. Nitroderivatives work as prodrugs being metabolized by NTR I of *Leishmania* spp. and the overexpression of this enzyme makes the parasites more sensitive to the action of these compounds. To test our hypothesis, *Leishmania infantum* promastigotes wild type (*Li*WT) and overexpressing a type 1 nitroreductase (*Li*NTR1) were incubated in the presence of various concentrations (0-128µM). It was possible to observe that the promastigotes transfected with the *Li*NTR1 were about four times more sensitive to the nitroderivative PHID121 than those transfected with the plasmid without the gene, a fact not observed for PHID40. Together, these results suggest that the nitroderivative PHID121 is a prodrug bioactivated by nitroreductases. **Supported by:**CIENTISTA DO NOSSO ESTADO - FAPERJ E-26/202.918/2018 **Keywords:**nitroreductases;nitroderivative;leishmanicidal activity.

TB-32 - **Antileishmanial activity of original amidoxime derivatives**

DA SILVA, M.B.C.[1]; LEON, O.L.A.[2]; CURTI, C.[2]; VANELLE, P.[2]; DE ANDRADE-NETO, V.V.[1]; TORRES-SANTOS, E.C.[1].
1. FIOCRUZ-RJ, BRA; 2. AIX MARSEILLE UNIVERSITÉ, FRA
E-mail: bellonicavalcante@gmail.com

The therapeutic arsenal against leishmaniasis is restrict and has several limitations. Our project concerns the synthesis of amidoxime derivatives presenting a 2,3-dihydrofuran heterocyclic scaffold that could offer a new option for the treatment of leishmaniasis. In this context, 2,3-dihydrofuran scaffold of the original structure bearing the amidoxime group was synthesized by a three-step procedure with different strategies: manganese (III) acetate radical oxidative cyclization by microwave irradiation, transition metal-catalyzed coupling reactions, amide bond formation and β-ketosulfone formation leading to heterocyclic derivatives. The amidoximes derivatives were subjected to *in vitro* evaluation of the cytotoxicity on murine peritoneal macrophages and antileishmanial activity on *Leishmania amazonensis.* We found two principal HITs, the 4-(5-benzyl-3-(4-fluorophenylsulfonyl)-5-methyl-4,5-dihydrofuran-2-yl)-*N'*-hydroxy benzimidamide (*L. amazonensis* promastigotes IC_{50}= 5.4 ± 1.0 µM, amastigotes IC_{50}= 7.9 ± 1.1 µM; CC_{50}= 102.0 ± 2.8 µM), and its methylate derivative in the benzyl group (*L. amazonensis* promastigotes IC_{50} : 5.6 ± 0.9 µM, amastigotes IC_{50}= 6.7 ± 1.2 µM; CC_{50}: 111.5 ± 7.3 µM) with a selectivity index (SI) of 12.9 and 16.6 respectively. Both with a better selectivity index than pentamidine which is 4.5 (IC_{50}= 1.9 ± 0.12 and CC_{50}= 8.5 ± 1.3 µM). From our ongoing work, we have previously reported that the presence of the dihydrofurane and amidoxime groups is necessary for the antileishmanial effect. Moreover, the influence of benzyl group substitution has been demonstrated. In conclusion, amidoxime derivatives presenting a 2,3-dihydrofuran heterocyclic scaffold are promising candidates for further biological evaluation. **Supported by:**FAPERJ - E-26/200.028/2021 **Keywords:** amidoximes; dihydrofuran; Leishmania amazonensis.

TB-33 - **Sterilization of PLGA-Amphotericin B microparticles by gamma irradiation**

DE LIMA, V.K.A.; SUZUKI, É.Y.; BORSODI, M.P.G.; BERGMANN, B.R.; SOUSA-BATISTA, A.D.J..
UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRA.
E-mail: vitoria@biof.ufrj.br

Cutaneous leishmaniasis (CL) is a neglected infectious disease caused by species of the genus Leishmania spp. and transmitted to humans by sandflies, causing ulcerated lesions. Although CL is not lethal, it generates morbidity, and the treatment is still based on multiple injections of toxic drugs, such as Amphotericin B (AMB), which is currently the most effective drug. Our group has demonstrated the local and single-dose efficacy of subcutaneous implants containing PLGA particles with AmB. The ultimate goal is to produce easily scalable particles that can enter the market to impact CL treatment. According to the Brazilian Pharmacopoeia, injectable formulations need to be sterile, so the objective of this work is to verify the sterility and physicochemical stability of the particles submitted to gamma irradiation. For this, the AmB particles were irradiated at a dose of 25 kGy with a Cobalt-60 gamma-ray source and then incubated in soy casein broth and TSA solid medium (Tryptic Soy Agar) for 14 days at 32º C. Analyzed size distribution (DLS), zeta potential, morphology (SEM) and thermal (DSC) and chemical (FTIR) stability. We did not observe microbial growth in the irradiated particles. The size and zeta potential were similar for irradiated and non-irradiated particles, being 2.5 µm and -24.6 mV; and 2.8 µm and -23.7 mV, respectively. In SEM, no damage was found on the surface of the particle. In DSC, no changes were observed in the thermal properties. In FTIR, no changes in chemical structure were observed either. It was seen that there were no changes in the efficacy and safety of amphotericin B. Thus, we conclude that there were no significant changes in the physicochemical characteristics of the particles. Gamma irradiation at a dose of 25 kGy proved to be effective for sterilization of the particles, without generating any morphological or physicochemical changes. **Supported by:**Vale -I.BIOF-22307 **Keywords:**PLGA;amphotericin B ;gamma irradiation.

TB-34 - **Evaluation of Antileishmanial Activity and Cytotoxicity of Chalcones-Thiosemicarbazones Hybrids Against *Leishmania infantum***

BARBOSA, R.C.F.[1]; BARBOSA, I.R.[1]; ECHEVARRIA, A.[1]; DE OLIVEIRA, M.C.C.[1]; TORRES-SANTOS, E.C.[2]; DE ANDRADE-NETO, V.V.[2].
1. UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO, RJ - BRA; 2. FUNDAÇÃO OSWALDO CRUZ (FIOCRUZ), RJ - BRA.
E-mail: rodrigocfbarbosa@gmail.com

Visceral leishmaniasis (VL) has the most severe clinical manifestations among leishmaniasis. The drugs used in its therapy have drawbacks such as high toxicity and resistance, which justifies the search for new drugs for the treatment. Recently, our research group evaluated hybrid compounds between chalcones and thiosemicarbazones against Leishmania amazonensis parasites. The results were promising and motivated us to prepare new hybrids and assess them against L. infantum, the species that causes VL. Antileishmanial activity and cytotoxicity of 13 hybrids were determined using the methodologies described by Andrade-Neto et al. All compounds evaluated against the L. infantum promastigote were active, presenting IC_{50} values in the range of 3.20-23.32 μM. The less active compounds have a strong electron-withdrawing group (IC_{50}: CT6 = 23,32 ± 3,56 μM; CT7 = 17,37 ± 2,61 μM; CT14 = 12,64 ± 3,75 μM), indicating that this electronic characteristic may be detrimental to the antileishmanial activity of the series evaluated. The other substances exhibited IC_{50} below 8 μM. Preliminary cytotoxicity assays revealed that the most active compounds against the parasite (CT1-5 and CT9-12) were also more harmful to isolated macrophages when tested at a concentration of 50 μM, with lethality above 50%. Interestingly, CT8, the most active hybrid against promastigotes (IC_{50} = 3,20 ± 0,87 μM), showed one of the lowest macrophage lethalities. Thus, the results obtained so far suggest that the presence of a fluorine atom in the meta position of the benzene ring possibly results in the best balance between antiparasitic activity and cytotoxicity. **Supported by:**CIENTISTA DO NOSSO ESTADO - FAPERJ E-26/202.918/2018 **Keywords:**Leishmania infantum;Chalcones-Thiosemicarbazones Hybrids;Visceral Leishmaniasis.

TB-35 - **Effect of Fluoroamodiaquine derivatives against *Leishmania amazonensis.***

DE ALMEIDA, R.N.P.[1]; VIANA, G.M.[2]; REZENDE, M.G.[2]; RODRIGUES, C.R.[2]; CABRAL, L.M.[2]; DA CUNHA JUNIOR, E.F.[3]; TORRES-SANTOS, E.C.[1].
LABORATÓRIO DE BIOQUÍMICA DE TRIPANOSOMATÍDEOS INSTITUTO OSWALDO CRUZ FIOCRUZ, RJ - BRA; 2. UFRJ, RJ - BRA; 3. UFRJ CAMPUS MACAÉ - RJ - BRA.
E-mail: raimundofarmas@gmail.com

Leishmaniasis is treated with a small arsenal of drugs, all of which have disadvantages in terms of toxicity, efficacy, price, or treatment regimen. Efforts have been made in the search for new and more effective leishmanicidal drugs. In this work, we evaluated in silico the physicochemical properties and ADMET profile of new fluoroamodiaquine (FAQ) analogs and their effect on *Leishmania amazonensis* and their toxicity on murine macrophages. Five FAQ analogs were designed and synthesized, using the tertiary amine to introduce a piperazine moiety plus a substituent (MR94: butyl, MR100: phenyl, MR102: 3-chlorobenzyl, MR104: 3,4-methylenedioxybenzyl, and MR106: tetrahydrofuran-2-carbonyl). The *in silico* evaluation was performed on the pkCSM platform. The best global result was obtained with MR106, as it does not infringe the Ro5. All of them have a high probability of intestinal absorption, with MR106 with the highest probability (92.7%). All show interaction as a substrate or an inhibitor of cytochrome P 450 enzymes, such as CYP3A4. The derivatives MR100, 102 and 104 are likely to have a positive AMES. All compounds showed a probability of hepatotoxicity, which is already known for amodiaquine derivatives; however, studies have shown that this characteristic does not remain in the analogs of the FAQ. The *in vitro* activity was evaluated by incubating promastigotes of *L. amazonensis* for 72h. The parasite viability was evaluated by the resazurin assay. For comparative purposes, we evaluated the activity of amodiaquine (AMQ) and miltefosine. Like AMQ (IC_{50} 11.6μM), all FAQ analogues were active, with the following IC_{50}s: 7.2μM (MR94), 4.2μM (MR100), 5.3μM (MR102), 2.8μM (MR104), and 44.7μM (MR106). All compounds and AMQ demonstrated similar cytotoxicity on murine macrophages, with CC_{50} around 35 μM. The derivatives with an aromatic ring showed greater potency on promastigotes, and despite a better in silico profile, tetrahydrofuran-2-carbonyl indicated a loss of potency. **Supported by:**FAPERJ **Keywords:** Drug Discovery; Leishmania; Fluoroamodiaquine.

TB-36 - **High-content screening of FDA-approved drugs in combination with benznidazole reveals potential anti - Trypanosoma cruzi agents.**

ALCÂNTARA, L.M.[1]; SAVOIA, M.N.[2]; VIEIRA, R.D.S.[1]; AMEIDA, R.R.[3]; NUNES, J.P.S.[3]; LOPES, I.[4]; KALIL, J.[3]; MANO, M.[4]; NETO, E.C.[3].
1. FACULDADE DE MEDICINA DA UNIVERSIDADE DE SAO PAULO - FMUSP, SP - BRA; 2. INSTITUTO DE MEDICINA TROPICAL, USP, SP - BRA; 3. LABORATÓRIO DE IMUNOLOGIA, INCOR, HOSPITAL DAS CLINICAS, FMUSP, SP - BRA; 4. FUNCTIONAL GENOMICS AND RNA-BASED THERAPEUTICS LABORATORY, CNC, UNIVERSITY OF COIMBRA POR
E-mail: lauramalcantara@outlook.com

Remoto Poster Session

,

Drug combinations and drug repurposing have emerged as promising strategies to discover novel therapeutics for Chagas diseases. We performed a fluorescence microscopy-based screening for anti-*T. cruzi* activity, in which a library of 640 FDA-approved drugs was tested isolated or combined with the reference drug benznidazole (BZN). From the primary screening, 30 drugs and 25 drug combinations were selected for further analysis. Among them, 3 drugs (oxcarbazepine, conduritol B and manidipine) and 2 drug combinations (clarithromycin + BZN and granisetron + BZN) were prioritized due to their superior antiparasitic activity and selectivity. The drug manidipine (MAN), a calcium channel blocker, was highly potent and efficacious (EC_{50} = 0.9 µM; maximum activity = 100%) and presented a selectivity index > 10. MAN efficacy was further confirmed in different host cells (U2OS, THP-1 and AC-16) and across distinct *T. cruzi* strains (Y, CL Brener and Colombiana). MAN displayed inhibitory effect on trypomastigote (EC_{50} = 14.5 µM) and epimastigote forms (EC_{50} = 4.8 µM), demonstrating a broad-spectrum profile. The drug also presented a fast-killing action, eliminating the infection after 24 hours of treatment. Furthermore, after removal of the drug, no intracellular amastigotes were observed, suggesting that MAN has a strong trypanocidal effect.
Regarding MAN+BZN combination, the fixed concentration of MAN at 1 µM potentiated the activity of BZN by 23-fold, allowing a significant reduction in the dose of the reference drug needed to achieve an effective response. Isobolographic studies demonstrated that BZN and MAN have an additive interaction (Σ FIC of 1.01).
In summary, our results demonstrate that MAN represents a promising drug candidate for Chagas disease treatment. Furthermore, our work reinforces the value of strategies based on drug combinations and repurposing to develop novel therapeutic strategies against Chagas disease and/or fuel targeted drug discovery efforts. **Supported by:**CAPES/FCT (Edital nº 28/2017 - 88887.156341/2017-00) **Keywords:**Chagas disease drug discovery;drug combination;drug repurposing/repositioning.