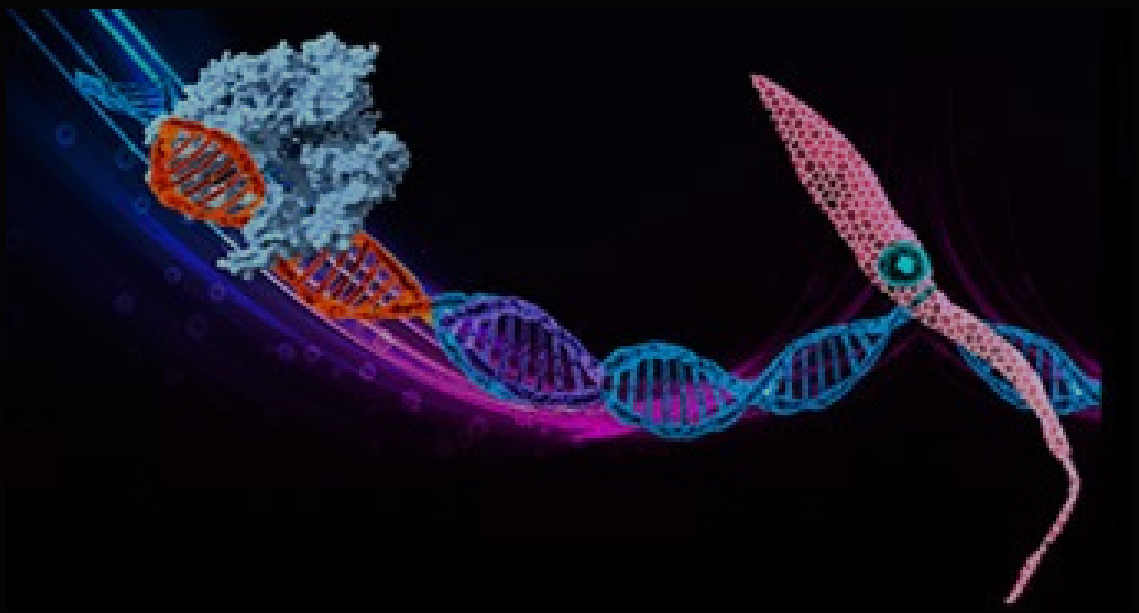
XXXVI Annual Meeting of the Brazilian Society of Protozoology

47th Annual Meeting of Fundamental in Chaga´s Disease

AUGUST 30th
SEPTEMBER 1st, 2021

ONLINE



SBPz

PROCEEDINGS

XXXVI Meeting of the Brazilian Society of Protozoology
XLVII Annual Meeting on Basic Research in Chagas' Disease

August 30th – September 1st , 2021
ONLINE

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Dear Participants,

On behalf of the Brazilian Society of Protozoology (SBPz) and the Organizing Committee, it is a great pleasure to welcome you all to the **XXXVI Meeting of the Brazilian Society of Protozoology** and the **XLVII Annual Meeting of Basic Research in Chagas´ Disease**, the so-called "**2021 Caxambu Meeting**".

Last year the Covid-19 pandemic surprised us and ended up preventing us from holding our traditional Annual Meeting. This year, still in a pandemic situation, we are conducting the Annual Meeting in a virtual format but honouring the spirit and the tradition of the meeting, we invited few keynote speakers and researchers from different countries and from Brazil to compose our scientific programme. In previous activities, that took place in April, May, and June, we organized three mini-courses and e Round Tables to discuss Immunology, Phylogenetic and In vivo cell culture of protozoa.

Despite the virtual format, we are keeping the students and young researcher's participation in virtual poster presentation that will culminate in discussion sections that we hope can be very full of challenges but resulting in productive participation of the audience. This way, we are guaranteeing the Zigman Brener Awards to be given to the best poster of different categories.

This year's Samuel Pessoa Award will be granted to Professor Santuza Maria Ribeiro Teixeira in recognition to her outstanding contribution to the field of protozoology.

Together with our sister society, British Society for Parasitology, we are also encouraging young researchers to discuss their problems in relation to the development of their careers and search for permanent positions in a Satellite Meeting that will allow them to create a favorable scenario for their work as scientists in research and teaching institutions.

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 distinct Board of Directors have assured the persistence of the SBPz, even in times like the actual pandemic scenario, guaranteeing the quality of the meeting and contributed to its international recognition. We would also like to acknowledge the financial support received THE COMPANY OF BIOLOGISTS (UK) and FAPESP.

So, we are very glad to welcome you all to this virtual "Caxambu Meeting", hoping that the scientific program covers expectations and provides high-level discussions as they have always occurred in face-to-face meetings.

São Paulo, August 30th, 2021

Lucile M Floeter Winter

President of SBPz

FOTO DA CAPA/COVER BOOK

The image represents the CRISPR technology of gene editing as a tool to study Trypanosoma and Leishmania parasites. The Cas nuclease is shown bound to parasite DNA. Once the DNA is cleaved in a precise location, insertion, modification or deletion of parasite sequences occurs and the parasite with its genome modified can be analised.

Dra. Santuza Maria Ribeiro Teixeira

FINANCIAL SUPPORT

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FAPESP

SPC-001 - **Genome studies of Trypanosoma cruzi: what have we learned about the parasite biology and host-parasite interactions**

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Trypanosoma cruzi, the causative agent of Chagas disease, is a protozoan pathogen that multiplies in the lumen of the insect midgut and within the cytoplasm of different types of mammalian cells. As a vector-borne disease, the parasite is disseminated among hundreds of mammalian species, besides humans, and within different species of insects belonging to the Triatominae family. To adapt to such wide variety of cellular environments including an obligate intracellular stage in the mammalian host, T. cruzi has developed sophisticated regulatory mechanisms to rapid change its repertoire of about 12,000 genes. Being an early divergent eukaryote, its genome is organized in polycistronic transcription units, which implies that gene expression regulation relies on post-transcriptional mechanisms mainly exerted by RNA binding proteins (RBPs). Together with a ratter limited number of research groups, our laboratory has been studying the T. cruzi genome and the molecular factors involved with gene expression and regulation. I will discuss the progress towards unraveling the T. cruzi biology as well as the mechanisms involved in host-parasite interactions, which have occurred thanks to parasite genome sequencing and studies involving gene manipulation approaches. I will also discuss more recent studies that begin to unveil molecular mechanisms used by the parasite to change its molecular composition, to subvert host defense mechanisms and manipulate host cellular programs so that it can benefit of living in several distinct environments. Besides characterizing genes encoding virulence factors, our studies have been focused on regulatory RBPs involved with changes in gene expression that are associated with parasite differentiation and the expression of virulence genes. These studies provide the framework necessary for the identification of new targets and the development of new strategies thar may result in more efficient methods to prevent and control a disease that still poses a significant threat to public health worldwide.
Keywords: Trypanosoma cruzi.genome.gene expression

CO-001 - **Defining the composition of Transitional Fibres in *Trypanosoma brucei***

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Transitional fibres are appendage structures surrounding the distal end of the basal body at the proximal end of the flagellum in *Trypanosoma brucei*. They are involved in connecting the flagellum to the flagellar pocket in Trypanosomes and there are analogous structures in mammalian centrioles – called distal appendages. There is very little understanding of the importance of these structures in Trypanosomes. The genome-wide protein localisation study, TrypTag, has been used to identify a cohort of proteins that localise to the basal body region of *Trypanosoma brucei*. Each protein has been endogenously tagged with an mNeonGreen fluorescent protein and co-localised in a cell line expressing SAS-6::mScarlet – a known basal body marker. A total of 35 putative transitional fibre proteins have so far been identified. Extracted cytoskeletons have been used in conjunction with automated image analysis to rapidly measure the dimensions of transitional fibre signals in thousands of cells and expansion microscopy was used to clearly identify the location on the transitional fibres. RNAi analysis of this cohort reveals the importance of proteins at the transitional fibres for flagellum morphogenesis.

CO-003 - **Inflammasomes, Leishmania and Autophagy: manipulation of host signalling pathways by Leishmania RNA Virus 1**

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Parasites of Leishmania genus have developed various strategies to overcome host immune response favoring its infection and development toward leishmaniasis. With an array of virulence factors, those parasites modify host macrophage signaling and functions. Depending on the species involved, visceral or cutaneous leishmaniasis will develop. Species such as Leishmania guyanensis and Leishmania braziliensis can be naturally infected with the endosymbiotic virus Leishmania RNA Virus 1. The presence of this virus was found to cause a particularly aggressive form of South-American mucocutaneous leishmaniasis. Data to be presented will report how the virus-containing parasites modulate innate immune sensors and signalling pathways including TLRs, TRIF, Type I IFN, autophagy and NLRP3 inflammasome networks that explain in part the exacerbated skin pathology caused by this particular parasite.
Supported by:FAPESP and CNPq **Keywords:** Leishmania.Inflammasome.LRV; Leishmania RNA Virus

CO-004 - **Combining transgenesis with paratransgenesis to fight malaria**

HUANG, W.[1]; KIZITO, C.[1]; VEGA-RODRIGUEZ, J.[2]; CHA, S.[1]; JACOBS-LORENA, M.[1].
1. DEPT MOLECULAR MICROBIOLOGY AND IMMUNOLOGY, MALARIA RESEARCH INST, BLOOMBERG SCHOOL OF PUBLIC HEALTH, BALTIMORE, MD - USA; 2. LABORATORY OF MALARIA AND VECTOR RESEARCH, NIAID, NIH, ROCKVILLE, MD - USA.

Malaria is among the deadliest infectious diseases and Plasmodium, the causative agent, needs to complete a complex development cycle in its vector mosquito for transmission to occur. Two promising strategies to curb transmission are **transgenesis**, consisting of genetically engineering mosquitoes to express anti-malarial effector molecules and **paratransgenesis**, consisting of introducing into the mosquito, commensal bacteria engineered to express anti-malarial effector molecules. Whereas both approaches restrict parasite development in the mosquito, it is not known how their effectiveness compares. Here we provide an in-depth assessment of transgenesis and paratransgenesis and evaluate the combination of the two approaches. Using the Q-system to drive gene expression, we engineered mosquitoes to produce and secrete two effectors – scorpine and the MP2 peptide – into the mosquito gut and salivary glands. We also engineered Serratia, a bacterium capable to spread through mosquito populations, to secrete the same two effectors into the mosquito gut. Human parasite *Plasmodium falciparum* oocyst and sporozoite intensity and mosquito prevalence were strongly reduced by expression of the anti-malaria effectors. Mosquito fitness, as measured by longevity, fertility, fecundity, and blood uptake, was not affected by the genetic modifications. Critically, substantially stronger reduction of P. falciparum development was achieved when transgenesis and paratransgenesis were combined. Most importantly, transmission of *P. berghei* from infected to naïve mice was strongly reduced. The combination of transgenesis and paratransgenesis promises to become a powerful tool to combat malaria. **Supported by:**AI127405 from the National Institute of Allergy and Infectious Diseases (NIAID) **Keywords:** Transgenesis.paratransgenesis.malaria transmission

CO-005 - **The arginine sensing pathway in *Leishmania***

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FACULTY OF BIOLOGY, TECHNION-ISRAEL INSTITUTE OF TECHNOLOGY, HAIFA - ISRAEL.

Parasitic protozoa of the genus *Leishmania* are obligatory intracellular parasites that cause human leishmaniasis. During their life, *Leishmania* cycle between the acidic phagolysosome of mammalian macrophages, where they reside as round amastigotes, and the mildly alkaline mid-gut of female sand flies, living as elongated promastigotes. A few years ago, my laboratory discovered that depletion of arginine from growth media induces *Leishmania* promastigotes and amastigotes to rapidly up-regulate expression and activity of the arginine transporter, AAP3. This arginine deprivation response (ADR) is also activated in intracellular amastigotes following macrophage infection and involves a mitogen activated protein kinase 2 (MPK2)-mediated signaling cascade. The *Leishmania* genome contains two identical copies of *AAP3* genes (AAP3.1 and 3.2), but only *AAP3.2* is responsive to arginine deprivation. Deletion of the *AAP3.2* locus, yielded mutants, which retain a basal level of arginine transport (from AAP3.1) that is not responsive to arginine deprivation. These mutants were avirulent to both macrophages and mice. Interestingly, ADR activity during infection influence the expression of SLC38A9, the host lysosome arginine sensor. Silencing the *SLC38A9* gene attenuated ADR in intracellular parasites and increased macrophage susceptibility to infection. My talk will provide an update on the ADR pathway and the crosstalk between host and parasite arginine sensing machinery.

CO-006 - **Disruption of Kharon and Trypanin genes and upgrade of the conditional knockout based on CRE-lox system in *Trypanosoma cruzi*.**

DAROCHA, W.D..
DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY UNIVERSIDADE FEDERAL DO PARANÁ, CURITIBA - PR - BRASIL.

Essential genes for parasite survival in mammalian host are key targets for pathogen attenuation or as target drug development. Kharon has been identified in other trypanosomatids as a cytoskeletal protein that acts on trafficking of some flagellar proteins and also crucial for parasite viability in mammalian stages (bloodstream in *T. brucei* and amastigotes in Leishmania). Trypanin, which was initially described as T Lymphocyte Triggering Factor (TLTF), functions as part of the dynein regulatory complex and it is lethal in the bloodstream form trypanosomes as shown by Trypanin silencing through RNAi. It is difficult to study gene essentiality in mammalian stages due to the lack of conditional knockout tools in *T. cruzi*. In this talk, it is going to describe our advances on developing an efficient CRE-lox system, a site-specific recombinase technology, widely used to generate conditional gene activation, deletions, inversions, insertions, translocation, and other genetic modifications. The strategy we have developed enabled the control of gene expression in tissue culture trypomastigotes, which are more difficult to transfect than epimastigotes. Towards the functional characterization of Kharon and Trypanin from *T. cruzi*, we have disrupted these genes using the CRISPR-Cas9 system and analyzed the phenotypic changes. Our results show that Kharon and Trypanin mutants behave differently from their counterparts in *T. brucei* or *Leishmania*. **Supported by:**Fundação Araucaria, UFPR, CNPq, and CAPES

CO-007 - **Advances on the impact of multiple blood meals on Leishmania inside the sand fly**

SERAFIM, T.D..
NIH, ROCKVILLE, MD - USA

Disease vectors transmit pathogens as they blood feed, and most vectors take multiple blood meals during their lifetime. A subsequent uninfected blood intake results in a remarkable effect to the Leishmania parasite inside the sand flies. Within 24h after blood feeding by a sand fly carrying a mature parasite infection, the metacyclic promastigotes, previously considered a terminally differentiated infectious stage, dedifferentiate to a leptomonad-like stage: the retroleptomonad promastigote. RNAseq analysis of retroleptomonads showed a distinct transcript expression profile to the other paraite promastigote stages inside of sand flies, validating it as a bona fide developmental form. Also, in the absence of a second blood meal, the small numnber of Leishmania parasites naturally acquired by feeding on an infected host are mostly lost before they develop into the infectious stage. This shows how crucial a second blood meal is and how important is the rise of retroleptomonads to increase the vectorial capacity of sand flies. These data reveal a novel and fundamental role for multiple blood meals in establishing the pathogen, and most importantly, in enhancing infectivity of the insect vector. These findings also place blood sources from other animals where infected sand flies would feed as a critical element in in the transmission of vector-borne pathogens. **Keywords:** Leishmania.Multiple Blood Meals.Vector competence

CC-001 - **From São Paulo to Bahia: a journey into the world of leishmaniasis, a neglected tropical disease**

DE OLIVEIRA, C.I..
FIOCRUZ BAHIA, SALVADOR - BA - BRASIL.

Leishmaniasis is a neglected tropical disease, linked to economically disadvantaged populations in tropical regions. It is a diverse human disease caused by many species of *Leishmania* parasites, which are transmitted by the bite of an infected sand fly. The clinical manifestation of leishmaniasis depends upon the species of the parasite and ranges from physical disfigurement to death if left untreated. The high prevalence of this disease is directly linked to the triad host–vector-parasite: *Leishmania* parasites manipulate the vertebrate immune system and exploits sand fly vector component, all favoring establishment of disease. Our laboratory has been involved in studying this interface, looking into the vertebrate immune response to sand fly salivary components and into the pathogenesis of Cutaneous Leishmaniasis, caused by the particularly vicious *Leishmania braziliensis*. I will present our findings in these areas, ranging from works conducted in experimental models to works in the field and how, in our opinion, we can move forward towards understanding the complexity of this relationship **Keywords:** Host-pathogen.sand fly saliva.ulcer

HP-001 - **MicroRNAs containing AAGUGCU seed sequence are important for Leishmania amazonensis infection in human THP-1 macrophages**

FERNANDES, J.C.R.; MUXEL, S.M.; FLOETER-WINTER, L..
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Leishmania amazonensis is a parasitic protozoan causing mainly cutaneous leishmaniasis in humans. Multiple mechanisms have been described regarding parasite-mediated rewiring of the immune response of host cell, especially macrophages, where *Leishmania*'s amastigotes multiply and establish infection. MicroRNAs (miRNAs) are small post-transcriptional regulators of gene expression leading to mRNA degradation or translational arrest by complementary binding of its seed sequence (nucleotides 2-8) with the 3' untranslated region of target mRNAs.
Although miRNAs have already been explored in multiple models of *Leishmania* infection, our approach is to compare their role in *L. amazonensis* infected human macrophages with the previously described one in murine macrophages. The expression of 84 immune response-related miRNAs was determined in human THP-1 derived macrophages infected with *L. amazonensis*. The upregulated miRNAs miR-372, miR-373, and miR-520d were further investigated since those miRNAs share the seed sequence AAGUGCU. This sequence is also present in the homologous murine miRNA mmu-miR-294, which was previously shown to target the nitric oxide synthase 2 mRNA. However, we could not find any significant modulation of mRNAs related to L-arginine metabolism in our model. Besides, the recognition site described in murine *Nos2* is mutated in humans.
Since the miRNAs of interest can potentially target similar pathways, we performed the functional inhibition assay with the individual miRNAs or in combination, to see if they could compensate the role of one, in case of the other being inhibited. We observed that inhibiting miR-372 or miR-520d, but not miR-373 alone reduced *Leishmania* infectivity while simultaneously inhibiting miR-372, miR-373 and miR-520d further decreased infectivity.
Here we report that a microRNA family, namely miR-372-373-520 is upregulated in *L. amazonensis* infected THP-1 macrophage correlating with infection susceptibility.
Supported by:FAPESP 2017/21906-9 **Keywords:** leishmaniasis.miRNA.regulation of gene expression

HP-002 - **MACROPHAGE SURFACE PROTEIN DISULFIDE ISOMERASE (PDI) ENHANCES INFECTION BY *Leishmania (L.) amazonensis***

PAIVA, G.C.M.; STOLF, B.S.. - USP, SÃO PAULO - SP - BRASIL

Leishmaniases are neglected parasitic diseases caused by parasites of the genus *Leishmania*, which are transmitted by female phlebotomine sandflies. Infections by different *Leishmania* species may lead to tegumentary or visceral complications in the vertebrate host. Chaperones present on macrophages 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 presence of PDI on the macrophage surface was associated with increased infection by *Leishmania chagasi*, a species associated with visceral leishmaniasis. The present study aimed to confirm the presence of PDI on the macrophage surface and evaluate its role on the infection by *Leishmania (L.) amazonensis*, a species responsible for cutaneous leishmaniasis. Cell membrane biotinylation assay was carried out to confirm the presence of PDI on macrophage surface. Bone marrow derived macrophages (BMDM) from BALB/c mice were then blocked with anti-PDI polyclonal antibody and infected with promastigotes of LV79 strain of *Leishmania (L.) amazonensis*. BMDM from transgenic mice overexpressing PDI (confirmed by *Western blot*) and wild type macrophages were also *in vitro* infected with *L. (L.) amazonensis*. *In vivo* imaging using M2269 La-LUC infection in transgenic mice overexpressing PDI and wild type mice is ongoing for comparison of lesion swelling and parasite load. The results of the biotinylation assay confirmed the presence 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 transgenic macrophages overexpressing PDI was higher than of the wild type counterparts. We expect that *in vivo* infections will lead to higher lesion swelling and parasite loads in PDI transgenic mice in comparison with the wild type counterparts. **Supported by:**(CAPES) 88887.360800/2019-00
Keywords: Leishmania (L.) amazonensis.PDI.Macrophage infection

HP-003 - **The *Trypanosoma cruzi* Enoyl-CoA hydratase, an enzyme involved in the catabolism of the branched chain amino acids, might be an interesting drug target**

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Trypanosoma cruzi, the causative agent of Chagas disease, depends on amino acids for many important biological functions. **Goals:** to increase our knowledge on the metabolism of branched chain amino acids (BCAA – Leu, Ile or Val) by *T. cruzi*. The enoyl-CoA hydratase (ECH) (also known as crotonase) catalyzes the fourth step of the BCAA metabolism. **Methodology:** In this work, the sequence encoding *T. cruzi* ECH (TcECH) was expressed and purified to determine its biochemical parameters. To assess the role of TcECH in the parasite biology, we obtained knockout mutants for both copies of *tcech* by using CRISPR/Cas9. We evaluated some phenotypic characteristics of these parasites. **Results:** The gene ablation resulted in the lack of crotonase activity as confirmed by measuring the enzyme activities in cell-free extracts. The knocked out parasites (D*tcech*) were submitted to nutritional stress in presence or not (control) of BCAA for 96 hours. Mutants and wild type parasites were resistant to starvation when incubated in the presence of Leu, but Ile and Val were deleterious for the mutants. Additionally, we found that D*tcech* parasites can complete the infection cycle in mammal cells at the same levels than control but have a decreased bursting of trypomastigotes from the infected cell when comparing with controls. **Conclusions**: Altogether, these data indicate that, the absence of crotonase activity induce the accumulation of a toxic intermediate in the presence of Ile and Val. Also the absence of TcECH affect the intracellular cycle of the parasite. Further experiments will allow us to better understand the role of the BCAA oxidation pathway for the biology of *T. cruzi*. **Supported by:** FAPESP 2017/14334-9 **Keywords:** Trypanosoma cruzi.Branched chain amino acids metabolism.Enoyl-CoA hydratase

HP-004 - **Identification and characterization of glycosomal iron transporters in *Leishmania***

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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 are able to 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, conserved genes encoding proteins addressed to the glycosomes, which are trypanosomatids unique organelles. Therefore, our goal is to identify and characterize genes involved in the transport of iron into the *Leishmania* glycosome. We initially performed the *in silico* analysis of the *Leishmania* transcripts significantly modulated by iron deprivation looking for those containing predicted glycosomal addressing signals (PTS1 or PTS2) and transmembrane domains. With this, we found 11 putative genes and selected 6 of them to confirm the subcellular localization of the encoded proteins. We cloned their ORFs into the *Leishmania* expression plasmids pXG-GFP+ and pXG-GFP2+, upstream or downstream of the GFP coding sequence. Thereby, we will confirm the subcellular localization of these proteins in *L. amazonensis* and characterize the overexpressing parasites regarding gene expression, replication and virulence. The identification of novel exclusive parasite' proteins may indicate potential targets for the development of new drugs for leishmaniases treatment. **Supported by:**FAPESP - 2019 nº 21185-5 - **Keywords:** Glycosomal.Iron.Transporters

HP-005 - **Characterization of Leishmania (L.) amazonensis Oligopeptidase B and its role in macrophage infection**

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Leishmania spp. are parasitic protozoa that cause leishmaniasis, a disease endemic in more than 98 countries, with more than 1 million new cases each year in the world. Symptomatic human infection may have different clinical forms, which are grouped into cutaneous and visceral. *Leishmania* promastigotes are transmitted to vertebrate hosts through the bite of female sandflies and differentiate into amastigotes within the host´s phagocytic cells. To survive the varied and hostile environments, the parasite has several virulence factors whose abundances vary according to the *Leishmania* species and the parasite stage. Oligopeptidase B (OPB) is a serine peptidase of the prolyl peptidase family (clan SC, family S9), characterized by the presence of a serine in the active site. This protein has been considered a virulence factor in different trypanosomatids. In fact, infections of mice with OPB-deficient *L. (L.) major* and *L. (L.) donovani* showed late lesion development, although little is known about the role of this protein, which is secreted in exosomes, in the parasite infection process. To fill the existing gaps, this project aimed to produce and characterize *L. (L.) amazonensis* recombinant OPB and to evaluate its effects on the infection of macrophages by this parasite species. We were able to produce and purify a recombinant soluble OPB in a bacterial system and used the recombinant protein to produce an anti-OPB serum in BALB/ c mice. We evaluated the activity and the enzymatic profile of the recombinant OPB, and our results indicate that the enzyme is active and has a profile similar to OPBs from other *Leishmania* species. Infection assays with BALB/ c bone marrow derived macrophages showed that OPB does not affect infection by *L. (L.) amazonensis* LV79 strain. Further assays are still required to determine the importance of this protein in *L. (L.) amazonensis* infection. **Supported by:**FAPESP- 2019/02391-3, CAPES - **Keywords:** L. (L.) amazonensis.Oligopeptidase B.Macrophage infection

HP-006 - **A map of PFR proteins and dissection of their functions in motility and assembly of the *Trypanosoma brucei* flagellum**

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Alongside the microtubule axoneme in many flagellated cells there are additional protein structures such as the paraflagellar rod (PFR) of *T. brucei*. The PFR has an intricate structure composed of three distinct domains (inner, middle and outer) that runs next to the axoneme within the flagellum and is important for flagellar beat regulation and cell motility. However, the specific contribution of each of the PFR domains to motility is unknown. The TrypTag project highlighted the complexity of the PFR with 146 proteins found in this structure. I determined by bioinformatics analysis, conservation patterns of these proteins across Euglenozoa and identified two major conserved sets of PFR proteins. The first set was conserved across Euglenozoa; whereas, the second set was conserved but not present in organisms with a reduced PFR, such as *Angomonas deanei*. A semi-automated analysis of TrypTag images, measuring the distance between the kinetoplast and the start of the PFR signal revealed a discontinuous start to the PFR and predicted the domain to which a PFR protein localised. These predictions were confirmed for a subset of 15 proteins by analysing the distance to known flagellum proteins. I analysed the function of these 15 proteins in cell motility and depletion of five of them disrupted motility, reduced cell growth and led to a ‘blob’ forming at the flagellum tip. The motility phenotypes were only observed in proteins present in the inner and very outer PFR domains and not present in organisms with a reduced PFR. However, for four of these proteins despite changes in motility, there was no obvious changes to the PFR2 localisation, suggesting these proteins are not required for PFR assembly. These results suggest that the motility function of the PFR can be separated from its assembly and that there are domains and sub-structures of the PFR with a specific role in motility. This is an important step in assigning specific functions to the individual PFR domains. **Supported by:**The Royal Society - NIF\R1\191618 - **Keywords:** Trypanosoma brucei.Paraflagellar Rod.functions

HP-007 - **P21 gene ablation of Trypanosoma cruzi induces changes in the virulence phenotypes**

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P21 is a protein expressed exclusively by *T. cruzi*. Studies using the recombinant form of P21 have shown that this protein plays essential roles at different steps of the infection of vertebrate host. Among the properties of P21 is the ability to induce signaling cascades in the host cell that results in the remodeling of the actin cytoskeleton, facilitating parasite internalization. Additionally, P21 interferes in the pathogenesis of Chagas disease, inhibiting angiogenesis, inducing inflammation, as well as controls amastigotes intracellular replication. Here we investigated whether the P21 deletion using CRISPR/Cas9 changes the parasite phenotypes involved in the infection of mammalian host. To obtain P21 knockout parasites, we have used the strategy of building a vector (pTREX/Cas9/sgRNA P21) carrying the Cas9 and the sgRNA-specific for the P21 gene. Transfected epimastigotes were selected with the antibiotics G418 and blasticidin, cloned by limiting dilution and four P21 KO clones were isolated. The absence of P21 mRNA and protein expression in the KO clones was verified by RT-PCR and immunofluorescence. Epimastigote growth kinetics showed that the P21 KO parasites have a slower growth rate than the wild-type parasites. In addition, incubation of HeLa cells with metacyclic forms for 4h demonstrated that the absence of P21 reduced the rate of invasion. However, the replication assay using HeLa cells infected with metacyclic forms showed that intracellular P21 KO amastigotes display increased replication rates when compared to control parasites. Together, our data show that P21 gene ablation alters the parasite growth, inhibits the invasion of metacyclic forms and increases the replication of intracellular amastigote. These results confirm that P21 may play a role in the development of non-proliferating intracellular amastigotes and may be responsible for the evasion of the host's immune system, constituting a crucial condition for the chronicity of the infection. **Supported by:**FAPESP, CNPq and CAPES - **Keywords:** P21 PROTEIN.CRISPR/Cas9.T. cruzi

HP-008 - **The importance of Sialic acids in Leishmania amazonensis and Leishmania infantum chagasi infection**

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Leishmania sp. are etiological agents of leishmaniasis, one of the most important parasitic diseases in the world. *Leishmania* promastigotes are covered by a dense glycocalyx composed by many glycoconjugates, which play an important role in *Leishmania* infectivity and survival. Sialic acids (Sias) are nine-carbon atoms sugars usually present as terminal residues of glycoproteins and glycolipids on the cell surface or secreted. The role of Sias in infections by protozoa such as *Trypanosoma cruzi* and *Leishmania donovani* was demonstrated in previous studies. The interaction between *Leishmania donovani* Sias and macrophage receptor Siglec-1 (Sialic acid-binding immunoglobulin-type lectins) contributes to the parasite's entry into the host cell. Due to the divergences among *Leishmania* species, the aim of this work was to evaluate the importance of Sias-Siglec-1 interaction in two endemic species in Brazil: *L. amazonensis* and *L. infantum chagasi*. For that, we treated parasites with sialidase, removing part of *Leishmania* Sias. *In vitro* infection assays using murine bone marrow-derived macrophages (BMDM) and cells of human lineage THP-1 showed that reduction of Sias decreased infection. These results were observed for both species and also for two different strains of *L. infantum chagasi*, MHOM/BR/1972/LD and MHOM/BR/2005/NLC, with the greatest impact of sialidase treatment for MHOM/BR/2005/NLC strain. We then analyzed Siglec-1 abundance in murine macrophages (BMDM) and human differentiated THP1 cells by flow cytometry. A low labeling was observed in BMDM, while more than 50% of THP1 cells expressed Siglec-1. Blocking of Siglec-1 had no impact in BMDM infection, perhaps due to the low abundance of this receptor in these cells. Blocking of Siglec-1 in THP1 cells is in course at this moment. Our results imply that sialic acid is important for *L. amazonensis and L. i. chagasi* infection and that the impact of sialidase treatment may vary between strains of the same species.
Supported by:CAPES, Processo: 88882.333062/2019-01 ; FAPESP **Keywords:** Leishmania.Sialic Acid.Macrophage infection

HP-009 - **The Cysteine Synthase enzyme plays an important role in the biological cycle of *Trypanosoma cruzi.***

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Trypanosoma cruzi can use amino acids as energy sources and to support several biological processes such as differentiation, resistance to stress conditions and host-cell invasion. Metabolites containing -SH groups (glutathione, trypanothione, cysteine, and some intermediates) are relevant to buffer the redox state of the different sub-cellular compartments of this organism. The cysteine synthase (CS) catalyze the second step in the *de novo* biosynthesis of cysteine, however, its role in redox homeostasis has been unexplored in *T. cruzi*. In this work, we identified a *T. cruzi* sequence encoding a functional CS (TcCS). We obtained partial knockout lineages for *TcCS* by using CRISPR/Cas9. TcCS-knocked out epimastigotes showed a lower proliferation rates and a diminished resistance to a short-time (30 min) exposition to 120 uM H_2O_2 when compared with controls constitutively expressing Cas9. When these parasites were submitted to nutritional stress in the presence (or not as a control) of 5 mM L-Serine, 5 mM OAS or different concentrations (0.2, 0.4 or 1 mM) of L-Cys we observed that: i. L-Cys concentrations over of 200 mM were lethal to the mutants after 48 hours and; ii. L-Ser and OAS contributed to the survival of both, mutants and wild type parasites to severe starvation. We found that *DTcCS* parasites had diminished their ability to differentiate to metacyclic trypomastigotes. When these metacyclic trypomastigotes were assessed for their ability to infect mammalian host cells, we observed an over rate of infection after 48 hours and an increment of trypomastigote bursting, however, most of these trypomastigotes showed severe morphological alterations when compared to the control. Altogether, these data indicate that cysteine has an important role during epimastigotes proliferation, metacyclogenesis and the infection of mammalian cells. Further experiments will allow us to better understand the role of the cysteine biosynthesis *de novo* for the biology of *T. cruzi*. **Supported by:**FAPESP - **Keywords:** Trypanosoma cruzi.Cysteine biosynthesis.TcCS

HP-010 - ***Trypanosoma rangeli* mucins overexpression induces reduction of parasitemia and alters parasite immune response modulation in mice**

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The mucin-like glycoproteins are known to be sialic acid acceptors on the surface of *Trypanosoma cruzi*. This huge glycoprotein family is involved in parasite attachment to the host cell and blocking lytic antibodies recognition, among other functions. Despite acting as virulence factors in *T. cruzi*, the mucins-like expression was identified in *Trypanosoma rangeli*, a non-pathogenic and non-cell invasive parasite of mammals. Here, we report the characterization and effects of overexpression of *T. rangeli* mucins (*Tr*MUC) in the host-parasite interaction. The *Tr*MUC family is composed of 93 genes classified into two groups: *Tr*MUCg and *Tr*MUCp that encode putative glycoproteins showing shared features with *T. cruzi* mucins I and II. BALB/c mice infected with *T. rangeli* overexpressing *Tr*MUCg or *Tr*MUCp show parasitemia reduction ($p < 0.0001$ and $p= 0.0146$, respectively) compared to control groups. Interferon γ (IFN γ) levels in the spleen of mice infected with wild type strain were reduced ($p=0.0018$), however normal IFN γ levels were detected in mice infected by *Tr*MUCg parasite ($p = 0.396$). A similar effect was observed on interleukin-6 (IL-6) levels in LPS-activated macrophages (RAW264.7 cells) in the presence of these parasites. While *Tc*MUCII expression by *T. rangeli* increases infection in mice ($p < 0,0001$) without altering cytokines secretion profile. Our observations indicate that *T. rangeli* can negatively modulate the host pro-inflammatory response *in vivo* and *in vitro* and, *Tr*MUC overexpression diminish this phenomenon. Also, unlike the *T. cruzi* mucins, *Tr*MUCs are little or non-glycosylated despite the functional glycosylation machinery in *T. rangeli*. This *Tr*MUC characteristic probably affects their ability to act as sialic acid acceptors, which match the absence of trans-sialidase activity by *T. rangeli*. An accurate *Tr*MUC glycoprofile could provide detailed information about the sialic acid acceptor role of this new genomic family in *T. rangeli*.
Supported by:CNPq, CAPES, FINEP, UFSC - **Keywords:** glycoproteins.mice infection.non-pathogenic parasite

HP-011 - **Neurodegenerative development during *Plasmodium berghei* ANKA infection is reduced by Amido-coumarin co-treatment**

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The *Plasmodium berghei* ANKA(PbA) infection in mice closely recapitulates many aspects of severe malaria in human including cerebral malaria. Coumarins are a class of secondary metabolites that are widely present in plants and exhibit several pharmacological, biochemical, and therapeutic effects, for example, anti-inflammatory and neuroprotective agents. Our aim was to investigate the potencial neuroprotection of an amido-coumarin compound in the treatment of experimental severe malaria. C57Bl/6 mice were inoculated with 10^5red cells parasitized with PbA and 3 days after infection (dpi) were orally treated daily with amido-coumarin compound MJM220(MJM-10mg/kg) alone and/or in combination with chloroquine (CQ-30mg/kg) once per day. Parasitemia, body weight, survival, clinical score, memory, and immune cell profiles in the spleen and brain at 5dpi by flow cytometry, were analyzed. Our results showed that the treatment with MJM220 alone reduced the parasitemia at 6° and 7dpi, and improvement of the clinical score from 8° to 12dpi when compared with infected untreated mice. The animals treated with MJM+CQ showed a reduction in parasitemia in all analyzed kinetics (3° to 47dpi). Treatments with MJM and MJM+CQ increased the survival of infected mice (80% and 60%;28°dpi and 47°dpi, respectively). 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: (i) in the brain, reduction in the numbers of macrophages, dendritic cells, CD4 and CD8T cells producing IFNγ, CD4T cells producing IL17 and IL10, and (ii) in the spleen, reduction in the number of neutrophils, CD4T cells produzing IFNγ or IL17, and increased number of CD8T cells producing IFNγ. Colletively, our results suggest that the compound MJM220, based on starch-coumarin, has a potential neuroprotective activity being a promising compound for co-treatment during severe experimental malaria. **Supported by:**CNPq (474971/2013-9)
Keywords: Coumarin.neuroprotection.antimalarial

HP-012- **Study of the cross-regulation between transport of iron and heme mediated by LIT1 and LHR1 in *Leishmania***

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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 parasitophorous vacuoles is known to have significant effects on parasite replication and virulence. This process requires the acquisition of essential nutrients such as iron and heme from the host since *Leishmania* does not have iron storage proteins and does not have heme biosynthesis capacity. Besides, iron and heme can be cytotoxic as they trigger the generation of free radicals in the presence of oxygen. *Leishmania*, therefore, must acquire heme and iron to survive in a hostile environment that restricts the availability of nutrients to the pathogen, a process called nutritional immunity. 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 one or both alleles of those transporters cause 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, LIT1 and LHR1. To begin, we used a single-step multi-fragment ligation strategy to assemble LIT1 targeting constructs. These constructs were transfected into *L. amazonensis* and LIT1 knockout clones were successfully selected. Currently, we are cloning LIT1 or LHR1 ORFs into the *Leishmania* expression vectors pXG-GFP2+ and pXG-GFP+. With these plasmids, we will generate mutant strains that overexpress LIT1 and LHR1 in the LHR1 single knockout and LIT1 knockout backgrounds, respectively. Those mutants will be evaluated regarding intracellular content of iron and heme, *in vitro* growth and virulence. 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:**FAPESP 2019/03861-3 **Keywords:** Leishmaniasis.Host-Parasite Interaction.Transition Metals

HP-013 - **Characterization of manganese transport mediated by the iron transporter LIR1 in *Leishmania***

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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. Recently, 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. Similar to iron, manganese is also a cofactor of several *Leishmania* enzymes, in particular arginase, which is essential for parasite replication and infection establishment in mammalian host. Considering the significance of manganese for *Leishmania* and the evidences 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*. For this, we quantified the total amount of parasite-associated manganese by inductively coupled plasma mass spectrometry (ICP-MS). Our preliminary findings revealed that LIR1 expression prevents manganese accumulation in *Leishmania.* Furthermore, western blot and arginase enzymatic activity assays indicate that *Leishmania*'s arginase is modulated by LIR1 expression. Therefore, our results corroborate a role of LIR1 in manganese homeostasis and its importance for parasite replication. Ultimately, we hope to evaluate the direct role of LIR1 in the transport of manganese across promastigotes plasma membrane. **Supported by:**FAPESP PROCESSO 2019/09715-9 **Keywords:** Leishmania.manganese.Arginase

HP-014 - **Alternatively activation of murine macrophage does not support infection by *Leishmania (V.) braziliensis***

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INTRODUCTION: Leishmania braziliensis is the main species responsible for American tegumentary leishmanias in Brazil. The control of Leishmania infection has been related to the classical activation of macrophages (cMo) that leads to parasite death. In the other way, macrophages polarized to alternative activation (AAMo) profile are described as susceptible to infection by *Leishmania spp*. The cytokines IL-4, IL-10 are important in polarizing different subtypes of AAMo. Thus, we aimed to evaluate *L. braziliensis* survival after infection of murine macrophage activated with INF-y and LPS, IL-4 or IL-10. METHODS: bone marrow-derived macrophages (BMDM) or Thioglycollate elicited peritoneal macrophages from C57BL/6 or BALB/c mice were stimulated with INF-y and LPS, IL-4 or IL-10 to evaluate nitric oxide production, arginase activity, and parasite load after infection with *L. braziliensis*. The number of infected macrophages and the parasite load were determined by light microscopy and by the recovery assay of promastigotes in culture. RESULTS: cMo cultured with INF-y and LPS produced high amount of NO (cMo 62.7 uM x CT 2.0 uM) but no increase in arginase activity, while AAMo cultured with IL-4 or IL-10 exhibited significant arginase activity (producing 538µg/mL and 515 µg/mL of urea respectively) and no NO production. In all situations there were significant decrease in the number of infected macrophages and amastigotes in infected macrophage cultured for 72 h. These results were confirmed by promastigote recovered in culture assay. CONCLUSION: These results suggest that control of *L. braziliensis* in murine macrophages occurs even with high arginase activity and independently of NO production. **Supported by:**FAPEG, CAPES, CNPq, INCT-IPH **Keywords:** Alternatively activation of macrophages.IL-4, IL-10.Leishmania (V.) braziliensis

HP-015 - **Glutamine is an important energy source for *Trypanosoma cruzi***

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Trypanosoma cruzi is the causative agent of American trypanosomiasis, also known as Chagas disease. Throughout its life cycle, *T. cruzi* alternates between mammalian hosts, humans among then, and the insect vector, a reduviid insect. Inside both hosts, the parasite needs to colonize distinct environments, such as the bloodstream and the cytoplasm of the cell in the mammalian host and the digestive tract in the insect vector. These environments present different nutrients availability and the parasite needs to adapt to the available nutrients. *T. cruzi* is able to use carbohydrates, amino acids and fatty acids as energy and carbon sources. In this work we demonstrate that *T. cruzi* is able to use glutamine (Gln) as an energy source. Gln can be completely oxidized to CO_2, fulfilling TCA cycle and sustaining the electron transport chain, contributing to ATP biosynthesis. In addition, exometabolomic analyses by ^{1}H-RMN showed that alanine and acetate are the main end products from Gln metabolism, demonstrating that Gln derived carbons are also feeding other metabolic pathways in epimastigotes. Taken together these data show that Gln is an important energy source for *T. cruzi*. **Supported by:**FAPESP, Processo número:2017/04808-3 **Keywords:** Trypanosoma cruzi.Bioenergetics.Glutamine

HP-016 - **Blood transcriptomics of human visceral leishmaniasis reveals insights into the long non-coding RNA and mRNA co-expression profile induced by Leishmania infantum infection.**

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Visceral leishmaniasis (VL) is an infectious, chronic and disseminated disease that can be potentially fatal if untreated. In Brazil, it is caused by *L. infantum* parasites transmitted by sand-fly vectors. Clinical outcomes of infection in leishmaniases are mainly mediated by host immune response and blood transcriptomics is powerful to assessing the molecular mechanisms of immunity. Thus, our aim was to perform analyses of bulk polyadenylated RNA-seq of VL patients (N=11) compared to non-diseased individuals: treated (T, cured VL patients; N=11), asymptomatic (A, resistant to disease; N=9) and healthy (H, control; N=9) in order to identify genes and molecular processes likely associated with immunopathophysiology that underlying the development of VL. Differential gene expression analysis, modular co-expression network analysis and gene features annotation used edgeR, cemitool and biomart R packages, respectively. Here, we focused on an integrated network analysis of mRNA with long non-coding RNAs (lncRNAs), which are key players of gene regulation during several pathological processes. We found 2,427 differentially expressed (DE) genes across all possible group comparisons, from which targeted approaches select a set of 1,512 genes to be explored by an integrated analysis of 147 DE lncRNAs (58 and 89 up- and down-regulated, respectively, in VL) and 1,263 DE mRNAs. Gene Set Enrichment Analysis identified co-expressed modules with significantly enriched pathways in the Over Representation Analysis, related mainly to “Hemostasis”, “Interferon Signaling”, “Cell Cycle Checkpoints”, “Neutrophil degranulation” and “Toll−like Receptor Cascades”. Co-expression networks based on highly correlated lncRNA-mRNA pairs (p-value < 5e-10) identified 6,060 positive and 1,351 negative pairs of correlations. Mining this lncRNA-mRNA correlated expression profile will provide insights into the lncRNA functions in immune-related processes triggered in visceral leishmaniasis patients. **Supported by:**FAPESP 2016/20258-0 **Keywords:** Leishmania infantum.blood transcriptomics.long non-coding RNA

HP-017 - **Gasdermin-D activation in response to Leishmania amazonensis induce a transient pore formation without cell death that promote NLRP3 activation and host resistance to infection**

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Leishmania is an obligate intracellular parasite the causes Leishmaniasis, a disease that affects millions of people worldwide. The parasites evade immune response by inhibiting cell death in many cell types. Here we investigated the role of Gasdermin-D (GSDMD, a pore-forming effector protein associated with pyroptosis) in *Leishmania amazonensis* infection and whether *Leishmania* inhibits pyroptosis-mediated cell death. We demonstrated that GSDMD is active in the early stages of infection, leading to low propidium iodide (PI) incorporation in live cells and low potassium efflux but without LDH release. Also, we observed that the *Gsdmd*$^{-/-}$ cells have less ASC puncta formation and secrets less IL-1β, suggesting that GSDMD pore formation contributes for the non-canonical activation of the NLRP3 inflammasome. Macrophages and mouse deficient in *Gsdmd*$^{-/-}$ mice were highly susceptible to *L. amazonensis* infection in vitro and in vivo, suggesting an important role of this molecule for non-canonical activation of NLRP3 and host resistance to infection. Altogether, our findings reveal that *Leishmania* infection trigger inflammasome-mediated GSDMD cleavage but not pyroptosis, and this process is very important for host immunity against these parasites.
Supported by:FAPESP **Keywords:** Leishmania.Inflammasome.GSDMD

HP-018 - **Genetic edition and drug free selection in Trypanosoma cruzi: new methodology for modifications by CRISPR/Cas9**

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Genetic engineering to tag genes using CRISPR/Cas9 technique is already well established in *T. cruzi* and usually inserts a drug resistance gene (DRG) so selection of the modified cells can be achieved. However a few problems arise from the need of drug selection such as: 1. the high price of drugs used for selection; 2. low number of antibiotics available which hinders the introduction of multiple modifications in *T. cruzi* genome simultaneously; 3. changes on the original gene 3' UTR that is important to mRNA stability and translational modulation. Considering these hindrances, we aim to validate a new methodology where target genes are tagged without insertion of a DRG and selection is performed by cloning process. For that purpose, epimastigotes constitutively expressing Cas9 and T7 RNA polymerase enzymes are transfected with two different PCR products: one for in vivo transcription of sgRNA by T7 RNA pol and another for homologous recombination. This latter is around 200 bp long which is composed of 3 copies of tag sequence (Myc, TY or HA) flanked by 30 bp of homology arms. We first attempted tagging the cell cycle kinase (CRK1) gene at the C terminal portion with Myc epitope, so epimastigotes were transfected with PCRs products and 24h later they were cloned. Seven clonal populations were obtained and their proteins were extracted and submitted to western blotting (anti-myc). 6 out of 7 clones showed a specific band around 35 kDa, which is the estimate mass of CRK1-Myc protein. Now we are trying to tag another two genes involved in cell cycle regulation: Cyclin 5 (HA tag at C terminal region) and Wee-1 (TY tag at N terminal region). Moreover, we also intend to modify two and three genes at once, so from one single eletroporation a monoclonal epimatigote population harboring three genes tagged can be obtained. So far we already can affirm that this methodology works and promises to be a valuable tool for *T. cruzi*. **Supported by:**FAPESP nº processo: 2019/17328-5 **Keywords:** T. cruzi.CRISPR/Cas9.Drug free selection

HP-019

Alterations and regulation of the mucosal barrier during mucin-*Giardia* interactions *in vitro*

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The extracellular secreted mucus prevent infection by the vast numbers of microorganisms that evolved a variety of mechanisms to cope with the mucosal barrier. In the case of *Giardia lamblia*, an extracellular protozoan parasite that colonizes the small intestine of humans and vertebrate animals, proliferative trophozoites have to penetrate or circumvent the mucus barrier to establish infection. In this study using in vitro co-cultures of *G. lamblia* trophozoites and intestinal epithelial cells (IECs), we have shown that mucin 2 (MUC2) and mucin 5AC (MUC5AC) are highly expressed in the human duodenum-derived adenocarcinoma cell line HuTu-80 in response to infection thus probably acts as a decoy to limit *Giardia* adherence to cell surface in the small intestine. By contrast, incubation of Caco-2 cells with trophozoites did not affected mucin production. Further, we evaluated whether trophozoites could promote degradation of mucins as a mechanism to control mucus abundance during infection. For this, trophozoites were incubated in a solution of isolated bovine submaxillary mucin or porcine gastric mucin and the expression pattern of MUC2 and MUC5AV were analyzed by Western blot. Our data show that trophozoites cleaves MUC5AC and totally degrades MUC2 independently of glycosylation as observed by Periodic-Acid Schiff (PAS) staining. Finally, we profiled phospho-signaling networks in IECs before and after exposure to trophozoites using the human Phospho-RTK array. IECs were incubated with trophozoites and the kinase activation patterns were compared to uninfected controls. CREB, Chk2, JNK, Akt1/2 and STAT2 phosphorylation increased in HuTu-80 cells exposed to *Giardia*, while phosphorylated p53 and STAT3 decreased. In Caco-2 cells infected with trophozoites the levels of phosphorylated p38a, EGF-R, MSK1/2 and STAT3 decreased however, no signaling cascades were activated in response to *Giardia*. Further studies are being conducted to confirm the observed signaling changes. **Supported by:**CNPq and FAPESP
Keywords: Mucus layer.intracellular signaling.giardiasis

HP-020 - **TNFR1, a conductor of inflammatory process against L. amazonensis**

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TNF is a cytokine involved in inflammation, tissue degeneration, tissue regeneration among other functions. TNF acts through two cognate receptors. TNFR1 seems to be more effective against intracellular parasites, including *Leishmania*. Studies about *L. major* elucidated the TH1-TH2 dichotomy. TH2 cells, through IL-4 and IL-13 secretion, active macrophages through the IL-4Rα leading to the metabolization of l-arginina by arginase I and production of polyamines, required to parasite replication. On the other hand, TH1 cells active macrophages through IFN-ɣ, which induces iNOS expression. iNOS, metabolizes L-arginine into citrulline and nitric oxide (NO), a leishmanicidal gas. Absence of TNF impairs signaling through its receptor and M1 activation, essential to control the parasite. However, what is the impact of TNFR1 in the infection by *L. amazonensis*? Our data reveal the importance of TNFR1 in iNOS and NO production expression by mononuclear phagocytes that leads to lesion control. Although expression of TNFR1 by wild type mice is not enough to eliminate the parasite, this receptor mediates control of parasite replication. Indeed, in *Leishmania* models of infection, sterile cure is not achieved. Recently NO was implicated in rewiring cells of the immune system by diminishing pro-inflammatory cytokine production that leads to the resolution phase of the inflammatory process. We observed a large inflammatory infiltrate, especially a larger number of lymphocytes at the site of infection in the absence of TNFR1. Furthermore, the few lymphocytes were regulatory T cells mainly, correlating with high levels of IL-10 at the chronic phase of infection. At the later time points macrophages present M2 phenotype, controlling immunopathology. 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.Macrophage polarization.Leishmania

HP-021 - **Role of STAT-1, STAT-3 and STAT-6 activation in modulating two *Trypanosoma cruzi* strains coinfection within human polarized macrophages M1 and M2**

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Introduction: Chagas' disease is classified as a neglected tropical disease by World Health Organization and affects approximately 8 million people worldwide. The disease is caused by the protozoan *Trypanosoma cruzi (T. cruzi)* and its genetic variability, host tissue tropism and host's immunological response are determinant factors for the disease progression and treatment. Transcription factors of JAK/STAT pathway presented trypanocidal activity, although parasite can modulate this pathway in order to thrive in intracellular environment. Moreover, coinfection with different *T. cruzi* strains is common at endemic areas. **Objective:** Evaluate the coinfection with two fluorescent-labelled *T. cruzi* strains (G-GFP and CL-DsRed), in comparison with single infection, in classic or alternatively activated human macrophages. **Methods:** Macrophages were derived from THP-1 cell line and polarized to M1 or M2 profiles to be further infected with trypomastigotes or extracellular amastigotes of *T. cruzi* (single or coinfection). We analyzed: % of infected cells, nitric oxide (NO), reactive oxygen species production and STAT-1, -3 and -6 phosphorylation levels. **Results:** Both strains were able to infect M0 macrophages, with different infectivity patterns. M1 demonstrated resistance and M2 susceptibility to infection of both strains and higher percentage of infected cells presented lower NO and vice-versa. STATs activation patterns were different among single and coinfection, demonstrating that both *T. cruzi* strains inside the macrophage elicited a new intracellular response. Moreover, we observed a paracrine effect in M2a non-infected cells next to infected cells with any strain, where M2a cells had some STATs dephosphorylated. **Conclusions:** Different activated STAT profiles were observed in coinfection in comparison with single infections; M2 profile may restrain the high inflammatory environment, being an important adjuster of tissue damage in response to *T. cruzi.* **Supported by:**FAPESP, Processo n. 2019/08933-2
Keywords: Trypanosoma cruzi.Coinfection.JAK/STAT Pathway

HP-022 - **Evaluation of different resins for ion exchange chromatography in the purification of T.evansi.**

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Trypanosoma evansi, a unicellular, hemoflagellate protozoan of the order Kinetoplastida, is morphologically indistinguishable from *T. brucei,* and characterized by a positively charged undulating membrane. Purification processes are important to isolate hemoparasites from other intrinsic components in the blood. In this context, the isolation of *T. evansi* is a process dependent on purification, which impacts diagnostic research. For this purpose, Diethylaminoethyl-cellulose (DEAE-Cellulose) is the resin commonly used to carry out ion exchange chromatography. The process of preparing the spine and the methodology to balance it present complications regarding the preparation period, in addition to its high cost. Seeking alternatives, our goal was to evaluate the purification efficiency and viability of *T. evansi* with porous silica chromatographic resins from Kopp Technologies (Purifica Y-N, Purifica Y-HONOH and Purifica Y-CNC3). Despite being intended for purification of several anionic compounds, it has never been tested for protozoa purification from blood samples. For this, we carried out the passage of samples through the three columns, where the efficiency of the processes in all resins was verified, through the observation of the protozoan viability by optical microscopy, Trypan blue and flow cytometry (propidium iodide). The results demonstrate the possibility of using these columns for the purification process of protozoa as a more affordable alternative, requiring further studies.
Supported by:FAPESC e CNPq **Keywords:** DEAE-cellulose.protozoan.viability

HP-023 - **Residence in a LAMP deficient intracellular environment interferes with biological and molecular characteristics of *Trypanosoma cruzi* trypomastigote forms**

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Trypanosoma cruzi invades non-professional phagocytic cells by subverting their membrane repair process, which is dependent on membrane injury and cell signaling, intracellular calcium increase and lysosome recruitment. Cells lacking Lysosome Associated Membrane Proteins 1 and 2 are less permissive to parasite invasion, however more prone to parasite intracellular multiplication. Several passages through a different intracellular environment can significantly change *T. cruzi*'s gene expression profile. Here, we evaluated whether one single passage through LAMP deficient (KO) or wild type (WT) fibroblasts could influence invasion ability of *T. cruzi* Y strain trypomastigotes in L6 myoblasts and WT fibroblasts. Parasites released from LAMP-2 KO cells (TcY-L2$^{-/-}$) showed higher invasion, calcium signaling and membrane injury rates when compared to those released from WT (TcY-WT) or LAMP-1/2 KO cells (TcY-L1/2$^{-/-}$) in L6 myoblasts. On the other hand, TcY-L1/2$^{-/-}$ showed higher invasion, calcium signaling and cell membrane injury rates compared to the others in WT fibroblasts. Albeit TcY-WT presented an intermediary invasion and calcium signaling rates in WT cells, they induced lower levels of injury, reinforcing that protein signaling may also have a significant contribution to parasite induced calcium signals. These results clearly show that parasites released from WT or LAMP KO cells are distinct from each other. Additionally, these parasites ability to invade the cell may be distinct depending with which cell type they interact to. Since these alterations most likely would reflect differences among parasite surface molecules, we also evaluated their membrane subproteome. Few protein complexes, membrane and secreted proteins were found regulated, such as some members of MASP, mucins, transialidases and gp63 proteins family, which may play an important role during parasite infection. **Supported by:**FAPEMIG/APQ-02974-17 **Keywords:** Trypanosoma cruzi infection.LAMP proteins.Proteomic profiling

HP-024 - **Assessing the infectivity of *Crithidia*-like parasites obtained from human visceral leishmaniasis cases through *in vitro* infection of macrophages**

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Visceral Leishmaniasis (VL) is caused by *Leishmania infantum* in Brazil and can be lethal if untreated. We have shown that some clinical isolates from VL cases in Sergipe, BR, do not belong to *Leishmania* and are phylogenetically related to *Crithidia*, a monoxenous genus considered non-pathogenic to humans. Here, we performed *in vitro* infection using mice bone marrow-derived macrophages (BMDM), J774 (murine) and THP-1 (human) cell lines. Cells were infected using *Crithidia*-like isolates, *L. infantum* and *C. fasciculata* strains during 24, 48 and 72 hours post infection (hpi). *Crithidia*-like isolates were able to infect all tested cell lines. The percentages of *Crithidia*-like infection using either J774 or THP-1 were, in average, 30% (24 hpi), 27.9% (48 hpi) and 22.15% (72 hpi) with an infection index of 57.5, 60.26 and 31.65, respectively. The highest porcentage of infection was obtained by *L. infantum* HUFS14 strain at 72 hpi (40% for J774 and 39.4% for THP-1) with an average infection index of 106,81. Likely, *C. fasciculata* was only phagocytosed by macrophages, rather than infected them, resulting in 5.6% and 4.3% of cells with internalized parasites at 24 hpi and 72 hpi and an infection index of 4.71 and 1.18, respectively. Interestingly, once BMDM were used, *Crithidia*-like showed a higher % of infection in all time courses compared to *L. infantum* PP75 strain. *Crithidia*-like infections reached up to 74% (24 hpi), while PP75 reached up to 28.25% (48 hpi). Infections using BMDM showed a better sensitivity of newly differentiated macrophages, corroborating that primary cells can be biologically more appropriated to use in infection assays. *In vitro* infections of *Crithidia*-like isolates will assist in the characterization of this new parasite. Moreover, infectivity assays will be performed using human peripheral blood mononuclear cells for evaluation of immune and microbicide responses, as well as gene expression induced by infection likely related to development of VL. **Supported by:**FAPESP grant 2016/20258-0, scholarship 2018/26799-9 and 2020/14011-8; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) **Keywords:** In vitro Infection.Visceral Leishmaniasis.Crithidia-like

HP-025 - **Participation of enolase enzyme on the infection of promastigotes of *Leishmania amazonensis***

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Certain molecules on the surface of *Leishmania* are pathogenic factors, as they control the different infection pathways of host cells. Apoptotic mimicry is one of such pathways, consisting of the ability to express surface molecules similar to those of apoptotic cells. Thus, the macrophage recognizes *Leishmania* and phagocytes it, without generating a harmful inflammatory response. Although phosphatidylserine phospholipid is one of the main markers involved in apoptotic mimicry, there are other molecules expressed by apoptotic cells capable of modulating the inflammatory response. The proteins of the glycolytic pathway are directed to the cell surface and, regardless of the intracellular roles in metabolism, these proteins in an extracellular context are candidates for immunomodulators. The objective of the work was to evaluate the participation of the enolase on the surface of *Leishmania amazonensis* during the development of the parasite *in vitro*. For this, the role of enolase in the infection of macrophages was evaluated, as well as the capacity of the enzyme in modulating the inflammatory activity of the macrophage. In addition, enolase expression kinetics was performed in metacyclic and procyclic forms. Results showed that the percentage of promastigotes expressing enolase on the surface varied throughout development *in vitro*. The correlation coefficient of the mean of *FSC* and the mean of the enolase for the strains LV-78 and Josefa showed a correlation between the expression of enolase and the size of the parasite. In addition, there was a decrease in the infectivity rate of macrophages when they were infected with promastigotes treated with anti-enolase antibodies. Thus, the data suggest that promastigotes of *Leishmania amazonensis* express enolase on its surface and there is a variation of this expression throughout the growth and development of the parasite *in vitro*, in addition to demonstrating that the enzyme has a role in the infection of macrophages.
Supported by:CAPES **Keywords:** Leishmaniasis.metacyclic.apoptotic mimicry

HP-026 - **Visceral infection model with *Leishmania amazonensis***

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Leishmaniasis is a group of clinical manifestations caused by parasites of the Leishmania genus, being the principal clinical manifestations: the cutaneou (CL), mucocutaneou (MCL) and visceral (VL). The determining factors of clinical manifestations are the species of the parasite and the host's immune status. The species *Leishmania amazonensis (L.a)* is mainly related to cases of CL, MCL and disseminated, but there are descriptions of cases of VL caused by the species. In animal models L.a is mainly related to studies of CL. The route of administration of the inoculum in animal models is important to determine the trajectory of infection. Intravenous infections (I.V) are considered the best models for the study of the chronic phase of VL and can be used the study the acute phase. Few studies are carried with model visceralizarion of L.a. Thus, the objective of our work is to standardize an intravenous infection model with a species of *L.a* for studies of visceralization of the parasite. To do so, we separated two groups of BALB / C mice and infected via I.V with infected macrophages (G1) and 106 amastigotes (G2). We evaluated the parasite load in bone marrow, spleen, liver and lymph nodes after 7, 21, 35, 49 and 64 days post infection (d.p.i.), with the limiting dilution method (LDA) after 7 days. As a result, we found that mice in group G1 first presented infection in the bone marrow at 7 d.p.i. and the spleen and liver were the last to be parasitized. In group G2 we only found parasite in the bone marrow at 7 d.p.i. We did not find linearity in parasite loads and infections over the weeks, this variability is justifiable by the route of inoculation. We conclude that infections by infected macrophages lead to consistent visceral infection in less time. More experiments are being carried out to confirm the route of infection and dissemination of the parasite, but this study shows promise for the study of visceral manifestation with species of *L. amazonensis*. **Supported by:**CAPES
Keywords: Leishmania amazonensis.Intravenous infections .visceralization

HP-027 - **Trypanosoma cruzi inositol phosphorylceramide synthase as a potential drug target for Chagas disease**

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Sphingolipids (SLs) are lipids characterized by the presence of sphingosine and are essential components in eukaryotic cell membranes. Inositol phosphorylceramide (IPC) is an SL present in several protozoa, but absent in mammals. In *Trypanosoma cruzi*, the etiologic agent of Chagas disease, IPC is synthesized by IPC synthase (TcIPCS), a trans-membrane protein expressed in all forms of the parasite. Thus, TcIPCS constitutes a potential target for the development of new chemotherapeutics. In this study, we aim to validate TcIPCS as a potential therapeutic target for Chagas disease, through the generation and characterization of genetically modified parasites. For this, we generated null TcIPCS mutants by CRISPR-Cas9 technology and showed that deletion affected epimastigote proliferation and metacyclogenesis, decreased in vitro infection capacity, intracellular replication of amastigotes and release of trypomastigotes from host cells. Interestingly, the supernatant from cells infected with the null mutants showed the predominance of extracellular amastigotes (EA). In order to validate the results obtained with the null mutants, we generated an add back cell line by transfection with a construct containing the IPCS gene with an HA tag. Western blot analysis using anti HA antibody suggested protein re-expression by the add-back parasites and infection experiments are being carried out to assess whether there was phenotype restitution in these parasites. Now we plan to perform biochemical assays to measure IPC synthesis in the cell lines generated in this study and confirm the involvement of IPCS in this process. Experiments with polyclonal antibody generated against an antigenic C-terminal fraction of the protein are also being carried out to analyze the expression profile of TcIPCS in the parasites. So far, we can conclude that TcIPCS, despite being important for fitness, is not an essential enzyme of T. cruzi, since it was possible to obtain null mutants for TcIPCS.

Supported by:FINEP **Keywords:** IPC synthase.Sphingolipids.Trypanosoma cruzi

HP-028 - **NTL25 inhibit the cellular proliferation on promastigotes of Leishmania spp and interact with trypanothione reductase.**

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Leishmaniasis is a neglected tropical disease caused by the parasite of the genus Leishmania presenting two main clinical forms: tegumentary and visceral. The treatments for leishmaniasis are toxic and expensive. This study evaluated the in vitro effect of the NTL25 on promastigotes of L. infantum and L. amazonensis, as well as demonstrated the interaction of this molecule with the Tripanothione Reductase (TR), an enzyme involved in the redox balance and important for the survival of the parasite. To evaluate the effect of NTL25 against promastigotes of L. infantum and L. amazonensis, these cells were incubated in the absence or presence with an increased concentration of NTL25 in a 96-well plate for 72h, after this, resarzurin was added and the fluorescence was measured at excitation wavelength 560 nm and emission 590 nm. Both species demonstrated an inhibition profile in a concentration-dependent manner presenting an IC50 value of 0,41 mM and 0,011 mM for L. infantum and L. amazonensis respectively. The parameter of ADMET was obtained using the pkCSM platform. The molecule demonstrates the percentage of 61,8% human intestinal absorption indicating oral administration, and not demonstrated to be mutagenic, carcinogenic, and hepatotoxicity. We also performed the interaction of the NTL25 with TR, using the oxidative form (2JK6) and reductive form (4ADW) of TR, analyzing the higher prevalence and lower energy conformations. The lower energy conformation presented a ΔG value of -10.81Kcal/mol and -10.71 Kcal/mol and an inhibition constant (Ki) of 11.83 nM and 14.21 nM for 2JK6 and 4ADW, respectively. The higher prevalence conformation presented a ΔG value of -9.87 Kcal/mol and -9.84Kcal/mol, and a Ki of 58.23 nM and 60.89 nM for 2JK6 and 4ADW, respectively. In conclusion, the NTL25 is a compound that can be orally administered, presenting an antileishmania effect and as a possible mechanism of action, the inhibition of TR, compromising the redox balance.

Supported by:CAPES - **Keywords:** Leishmania.Flavonoid.Treatment

HP-029 - **Treatment with Suboptimal dose of Benznidazole Mitigates Immune Response Molecular Pathways in Mice with Chronic Chagasic Cardiomyopathy**

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Chronic chagasic cardiomyopathy (CCC) is the most frequent and severe form of Chagas disease, a neglected tropical illness caused by the protozoan *Trypanosoma cruzi*. Although efforts have been made to understand the signaling pathways and molecular mechanisms underlying CCC, we still lack information on the immunological signaling pathways regulated by the etiological treatment with benznidazole (Bz). In experimental CCC, Bz combined with the immunoregulatory agent pentoxifylline (PTX) has beneficial effects on CCC. C57BL/6 mice chronically infected with *T. cruzi* Colombian strain (TcI) showing electrocardiographic abnormalities were submitted to treatment with suboptimal dose of Bz or Bz+PTX from 120 to 150 days post-infection. Electrocardiographic alterations and heart parasite load were beneficially impacted by Bz and Bz+PTX. RT-qPCR TaqMan array was used to evaluate the expression of 92 genes related to the immune response in RNA extracted from heart tissues. In infected mice, 30 genes were upregulated and 31 were downregulated. Infection upregulated the cytokines IFN-γ, IL-12b, and IL-2 (126-, 44-, and 18-fold change, respectively) and the T-cell chemoattractants CCL3 and CCL5 (23- and 16-fold change, respectively). Bz therapy restored the expression of genes related to inflammatory response, cellular development, growth, and proliferation, and tissue development pathways, linked to the cardiac remodeling processes inherent to CCC, thus mitigating the Th1-driven response found in vehicle-treated infected mice. The combined Bz+PTX therapy affected pathways related to the modulation of cell death and survival and organismal survival, supporting that this strategy may mitigate the progression of CCC. Altogether, our results contribute to the better understanding of the molecular mechanisms of the immune response in the heart tissue in chronic Chagas disease, and reinforce that parasite persistence and dysregulated immune response underpin CCC severity. **Supported by:**CAPES, CNPq (311539/2020-3, BPP 306037/2019-0) and FAPERJ (JCNE, E-26/203.031/2018, CNE, E-26/ 210.190/2018) **Keywords:** Chagas disease.Immune response.Benznidazole

HP-030 - **Modulation of miR-145-5p and miR-146b-5p levels is linked to reduced parasite load in the H9C2 cardiomyoblast cells infected with *Trypanosoma cruzi***

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The microRNAs miR-145-5p and miR-146b-5p are involved in the regulation of genes related to immune response and inflammatory processes. In Chagas disease, miR-145-5p and miR-146b-5p have been shown to be downregulated and upregulated, respectively, in the heart tissue of acutely *Trypanosoma cruzi*-infected mice. Initially, we established an *in vitro* experimental model using the H9C2 rat cardiomyoblast cell line infected with the Colombian *T. cruzi* strain to investigate the effect of parasite infection on the regulation of miR-145-5p and miR-146b-5p levels by the host cell. Later, we explored the effects of treatment of *T. cruzi*-infected H9C2 cells with the trypanossomicidal drug Benznidazole (Bz) alone or combined with the immunoregulator Pentoxifylline (PTX) on parasite load and expression of these miRNAs. The infection of the H9C2 cells with trypomastigote forms allowed parasite cycle with intracellular forms multiplication and trypomastigote release. After 48 and 144 hours of infection, we detected upregulation of miR-145-5p and miR-146b-5b levels. Addition of 3 μM and 10 μM of Bz 48 hours after infection was able to reduce parasite load but did not interfere with miR-145-5p and miR-146b-5p levels. On the other hand, associated Bz+PTX treatment decreased the levels of both microRNAs, restoring the same levels detected in the non-infected H9C2 cells. The use of miR-145-5p and miR-146b-5p mimic/inhibitor systems before the H9C2 infection significantly decreased parasite load, 72 hours after infection. In parallel, when H9C2 cells were treated with miR-145-5p and miR-146b-5p mimic/inhibitor 48h after infection, all the used systems reduced the parasite load. Taken together, our results show the potential of miR-145-5p and miR-146b-5p to be further investigated as biomarkers of parasite control and tools to identify therapeutic targets to be used as adjuvant to etiological treatment in Chagas disease. **Supported by:**CAPES, CNPq (311539/2020-3, BPP 306037/2019-0) and FAPERJ (JCNE, E-26/203.031/2018, CNE, E-26/ 210.190/2018) - **Keywords:** Chagas disease.microRNA.Benznidazole

HP-031 - **Development of a qPCR method for parasite load quantification of *Leishmania spp***

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Leishmaniasis is a tropical and subtropical endemic disease caused by parasites of the *Leishmania* genus. Leishmaniasis present some clinical manifestations which depend on the infecting *Leishmania* species and the host immune response. The disiase can be classified into two types: tegumentary and visceral leishmaniasis. Since the isolated symptoms from leishmaniasis are not enough for the diagnosis, which is based on the visualization of amastigote forms of the parasite in biopsy tissue. Thus, the diagnosis confirmation needs other techniques with greater sensibility and capacity to identify infective species. The quantitative Polymerase Chain Reaction (qPCR) method presents a greater detection sensibility and it is capable of quantifying the *Leishmania* parasite load. In addiction, this method has shown some technical advantages such as speed, reduced risk of contamination and possibility to quantify the parasite. Therefore, we standardized a qPCR assay to quantify the parasite load of *Leishmania braziliensis* in infected bone marrow-derived macrophages, as well as in infected C57BL/6J and BALB/c mice tissues. This assay demonstrate to be able to obtain the parasite quantification from a standard curve of the number of parasites by the Ct (Threshold Cycle). Once the target gene is conserved among *Leishmania* species, the method developed could be to applied to identify and quantify other *Leishmania* parasites. Moreover, the species differentiation relies on the use of specific probes containing distinct fluorophores. So, the standardization of this method can be applied to diagnosis, treatment efficacy and epidemiological studies as well as vaccines assays.
Supported by:CAPES: 88887.517727/2020-00 **Keywords:** Parasite load.qPCR assay for Leishmania parasites.Molecular diagnosis

HP-032 - **Leishmania infection inhibits macrophages migration in a three-dimensional environment**

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Leishmaniasis results in a wide spectrum of clinical manifestations, ranging from skin lesions at the inoculation site to disseminated lesions to internal organs, such as spleen and liver. The ability of *Leishmania*-infected host cells to migrate may be important to lesion distribution on the host and the dissemination of disease. However, the mechanisms involved in parasite dissemination and the accompanying role played by host cells remains poorly understood. Previously published work has shown that *Leishmania* infection inhibits macrophage 2D migration by altering actin dynamics and impairing the expression of proteins that function in plasma membrane–extracellular matrix interactions. However, cell migration *in vivo* mostly occurs in 3-dimensional (3D) environments. The present study aimed to investigate the migration of macrophages infected by *Leishmania* and the mechanisms involved in this process, using a 3-dimensional environment. Following the infection of bone marrow-derived macrophages (BMMF,) by *L. amazonensis*, *L. braziliensis*, or *L. infantum*, cellular migration, the formation of adhesion complexes and actin polymerization were evaluated. We found that BMMF 3D amoeboid migration was inhibited following *Leishmania* infection. Reduced expression of proteins involved in adhesion complex formation and altered actin dynamics were also observed in *Leishmania*-infected BMMF. Taken together, our results show that *Leishmania* infection inhibits BMMF 3D amoeboid migration by altering actin dynamics and impairing the expression of proteins that function in plasma membrane extracellular matrix interactions.
Supported by:FAPESB, Nº 9092/2015 **Keywords:** Macrophages.3D Migration.Leishmania

HP-033 - **Single Nucleotide Polymorphisms of two *Trypanosoma cruzi* genes encoding truncated type-I nitroreductases (TcNTR-1 and TcOYE): *in vitro* resistance to Nitro-heterocyclic drugs and treatment failure**

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Currently, the two drugs approved for Chagas disease (CD) therapy, Benznidazole and Nifurtimox, have serious limitations, namely: prolonged treatment regimens, substantial side effects and failures. A relationship between parasite genetic diversity and drug susceptibility has been observed, but its epidemiological significance remains unclear. To gain information on this issue, we sequenced two *T. cruzi* genes involved in *in vitro* resistance to nitro-heterocyclic drugs. The nucleotide sequences of type I nitroreductase (TcNTR1) and old yellow enzyme (TcOYE) genes were identified in 76 strains and isolates belonging to different discrete typing units from vectors, reservoirs and patients (oral CD, chronic, congenital and CD reactivation) with variable degree of susceptibility. The genes showed ORFs of 941 bp (TcNTR1) and 1120 bp (TcOYE), encoding proteins of 313 and 373 amino acids (aa), respectively. The deduced aa sequences of TcNTR1 and TcOYE exhibited 94% and 72% of average homology among samples. Among TcNTR sequences, ten SNPs were detected, the most recurrent SNPs were G/A transitions (n=13 isolates) at aa positions 18, 239, and 243, which generated premature stop codons at the hypothetical carboxyl-terminal FMN binding site. The TcOYE gene sequence analysis showed six SNPs that also originated premature stop codons; the most frequent SNPs were deletions at positions 28 and 38 that generated a UGA codon at aa 27 (n=7 isolates) and deletions at ntds 180, 504, 519, 560, 712, 735, 750 that led to a UAA codon at aa 253 (n=13 isolates). These stop codons affected positions located at the hypothetical FMN and substrate binding sites. All these aa changes correlated with reference strains already characterized as resistant phenotypes plus 15 new isolates that can be classified as putative resistant to nitro-heterocyclic drugs at a genetic level, some of them isolated from oral CD patients infected with Tc I strains refractory to treatment. **Supported by:**ERANET LAC HD 328 - **Keywords:** Trypanosoma cruzi.drug resistance genes .single nucleotide polymorphism

HP-034 - **Natural Populations of *Trypanosoma cruzi* in Oral Chagas Disease Patients Refractory to Benznidazole Chemotherapy: Molecular characterization through 9 years of Follow-Up**

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We have characterized the genetic profiles of natural populations of *T. cruzi* present in patients with poor response to etiological treatment, affected by the outbreak of Oral Chagas Disease (OCD) in a rural school at the community of Chichiriviche de la Costa, Venezuela. Six hemocultures (HCs) and 102 venous blood samples were obtained from twenty-nine OCD patients at the time of diagnosis and along nine years of Post-treatment (Tx) follow-up. The IgG serology, *T. cruzi* discrete typing units (DTU), satellite DNA-qPCR parasitic loads, and minicircle signatures (Ms) were determined in all samples. The serological titers and parasitic loads changed after treatment, with a significant decrease of IgG titers (Spearman's r value= -0.961) and median parasite loads from 2.869 [IQR = 2.113 to 3.720] to 0.105 [IQR = -1.147 to 1.761] log10 par eq./mL at Pre-Tx and Post-Tx, respectively, suggesting infection evolution from acute to chronic phase, without seroconversion or parasitological suppress. All patients were infected with *T. cruzi* DTUI populations. The median of the Jaccard similarity coefficient in Pre-Tx samples was 0.775 [IQR = 0.708 to 0.882], decreasing in genetic variability towards the end of follow-up (Mann-Whitney U test p= 0.0031). Interestingly, no Post-Tx Ms was identical to its Pre-Tx counterpart population in the same patient, revealing a selection of parasite subpopulations between the primary infection and Post-Tx. Parasitic populations isolated from HCs showed a lower number of bands in the Ms with respect to the signatures obtained directly from the patients' blood samples, demonstrating a process of parasitic selection and reduction of the population variability that initially infected the patients. Decrease of parasitic loads after Tx as well as Pre and Post-Tx intra-TcI diversity might be a consequence of both, a natural evolution of the acute infection to the chronic phase and persistence of refractory populations due to Tx selection.
Supported by:NHEPACHA NETWORK **Keywords:** ORAL CHAGAS DISEASE.Trypanosoma cruzi I genetic polymorphism.treatment failure

HP-035 - **The asparagine synthetases from Trypanosoma cruzi: from coding genes to function.**

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In view of the absence of an efficient prophylactic treatment for Chagas disease, in order to identify new targets and therapeutic approaches, several studies have been developed aiming to deepen the knowledge of the biology of Trypanosoma cruzi, the etiological agent of this parasitic burden. In this sense, the amino acid metabolism has come up as a rational study proposal, given its paramount role in several aspects of the parasite biology. Besides being precursors of protein biosynthesis, amino acids provide carbons and energy and are involved in several critical aspects of the biology of T. cruzi, such as cell differentiation, host cell invasion, cell cycle regulation and stress resistance. A specific enzyme involved in aminoacid metabolism, the asparagine synthetase (AS), catalyzes the production of asparagine using aspartate as substrate and glutamine or ammonia as a nitrogen source. In this work we propose the hypothesis that this enzyme is involved in the adçministration and availability of important metabolites derived from aspartate, glutamine, asparagine and glutamate, and as well as in the acceptance and transference of amino groups and concomitant resistance to NH_4+ in T. cruzi. We start by identifying the genes coding for putative asparagine synthetases in T. cruzi and the subsequent cloning and heterologous expression of the recombinant proteins with the aim of performing a biochemical characterization of the enzymes. We also produced mutants with deleted sequences for the respective genes using the CRISPR-Cas 9 system, which will provide information about the functional role of these enzymes in different aspects of the biology of T. cruzi.
Supported by:FAPESP - Fundação de Amparo à Pesquisa do Estado de São Paulo. Processo N° 2016/06034-219 **Keywords:** Trypanosoma cruzi.asparagine synthetase.Metabolism

HP-036 - ***Leishmania*-Induced Dendritic Cell Migration and its Potential Contribution to Parasite Dissemination**

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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*. 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. In addition, we evaluated the role of LPG, a key molecule involved in *Leishmania*-host cell interaction, in the migration of human dendritic cells infected by *Leishmania*. 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. Also, we observed reduced migration in hDC infected by *L. infantum* lpg2 knockout when compared to those infected with the wildtype parasites. Reduced expression of proteins involved in adhesion complex formation was also found in hDC infected by *L. infantum* lpg2 knockout. Together, our results suggest that hDCs play an important role in the dissemination of *Leishmania* parasites in the vertebrate host and that LPG plays a role in the modulation induced by these parasites in these cells. **Supported by:**Fapesb 9092/2015 **Keywords:** Dendritic cell; Leishmania.Migration; Dissemination.LPG

HP-037 - **Protein ANXA1 during an experimental infection by *T. gondii: in vitro* and *vivo***

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INTRODUCTION: Toxoplasmosis, caused by *Toxoplasma gondii* (Tg), is a public concern owing mainly to its neurotropic nature. Tg is an obligatory intracellular parasite, and macrophages and glial cells are crucial controlling this infection/inflammation. **AIM:** Annexin A1 (ANXA1) is a pro-resolving and anti-inflammatory protein, and its role during Tg infection is unknown and was evaluated here. **MATERIALS E METHODS:** Peritoneal macrophages and glial cells from Balb/c (WT) and ANXA1 knockout(KO) mice were infected with Tg RH strain, *in vitro*. WT and ANXA1 KO mice were infected or not with Tg ME49 strain and weight loss and survival was monitored. Moreover, the peritoneal leukocytes phenotype was distinguished and quantified during infection. **RESULTS:** Macrophages and glial cell ANXA1 KO, *in vitro*, increased the tachyzoites number compared to WT cells. *In vivo,* ANXA1deficiency increased the *T. gondii* infection susceptibility, but not weight loss, associated with increased number of brain cysts when compared to WT counterparts. Moreover, a reduction number of lymphocytes, but not macrophages and neutrophils, was found in the peritoneal cavity of infected ANXA1 mice when compared to WT. **CONCLUSION:** Our data suggested an important role of ANXA1 during Tg infection regulating lymphocytes migration and/or activation, and macrophages and glia cells antitoxoplasma activity.
Supported by: Cnpq, Fapemig, Capes **Keywords:** Toxoplasma gondii.Annexin A1.Immune response

HP-038 - **ANXIETY AND DEPRESSIVE-LIKE BEHAVIOR IN LONG-TERM Toxoplasma gondii INFECTION OF C57BL/6 MICE ARE ASSOCIATED WITH DISRUPTION OF THE BLOOD BRAIN BARRIER AND INCREASE IN SITU AND SYSTEMIC CYTOKINE EXPRESSION**

BARRIOS, L.M.C.[1]; PINHEIRO, A.P.[1]; GIBALDI, D.[1]; DA SILVA, A.A.[2]; SILVA, P.M.R.E.[1]; ROFFE, E.[3]; SANTIAGO, H.D.C.[4]; GAZZINELLI, R.T.[4]; MINEO, J.[5]; SILVA, N.[5]; LANNES-VIEIRA, J.[1].
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Toxoplasmosis is caused by the protozoan *Toxoplasma gondii*, a mandatory intracellular parasite. This illness is of medical importance due to the high prevalence worldwide, the ability to cause abortion and malformations in fetuses, and neurological alterations in immunocompromised persons, as HIV-infected individuals. In chronically infected immunocompetent individuals, this parasite forms tissue cysts mainly in the brain. In addition, *T. gondii* infection has been related to mental illnesses as schizophrenia and depression. Here, we evaluated the kinetics of behavioral alterations in chronic infection, assessing anxiety and depressive-like behavior, and their relationship with the number of parasite cysts in brain, blood-brain-barrier (BBB) integrity, and cytokine status in the brain and serum. Adult female C57BL/6 mice were infected (gavage, 5 cysts, ME-49 type II *T. gondii* strain) and analyzed as independent groups at 30, 60 and 90 days after infection (dpi). Anxiety and depressive-like behavior were detected in the early (30 dpi) and long-term (60 and 90 dpi) chronic *T. gondii* infection, in a direct association with the presence of parasite cysts and linked to BBB disruption. The behavioral alterations paralleled the upregulation of expression of tumor necrosis factor (TNF) and CC-chemokines (CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β and CCL5/RANTES) in the brain tissue. In addition, the behavioral changes paralleled with peripheral blood levels of interferon-gamma (IFNγ), TNF and CCL2/MCP-1, at 30 and 60 dpi. Our data suggest that the persistence of parasite cysts in the CNS induces increase in cytokine expression and sustained BBB disruption, thus allowing leakage of cytokines of systemic circulating plasma into the brain tissue. Therefore, these factors may contribute to anxiety and depressive-like behavior in chronic *T. gondii* infection.
Keywords: Behavioral tests.ME-49 strain.Neuroinflammation

HP-039 - **HYPERACTIVITY IN A MURINE MODEL OF LONG-LASTING CHRONIC Toxoplasma gondii IS REFRACTARY TO SULFADIAZINE AND PYRIMETHAMINE THERAPY**

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1. FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - RJ - BRA; 2. FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRA; 3. UNIVERSIDADE FEDERAL FLUMINENSE, NITEROI - RJ - BRA; 4. UNIVERSIDADE FEDERAL DE UBERLÂNDIA, UBERLÂNDIA - GO - BRA

Toxoplasma gondii, an intracellular protozoan, is the etiologic agent of toxoplasmosis, an infectious disease of medical importance. The *T. gondii* protozoan is found worldwide and currently one third of the world population is seropositive. The parasite has tropism for the central nervous system (CNS), where it remains for long periods influencing the behavior of the host. In fact, infection by *T. gondii* has been related to mental illnesses such as schizophrenia, bipolar disorder, obsessive-compulsive disorder, and other behavioral abnormalities. The aim of the present study was to determine whether the presence of *T. gondii* cysts in the CNS leads to hyperactivity in chronically infected animals. For that, 4-6 weeks old C57BL/6 mice were infected with the cystic form of the ME-49 type II *T. gondii* strain and evaluated at 30-, 60- and 90-days post-infection (dpi). Later, we tested the role of parasite in the behavioral alterations. Thus, chronically infected mice were orally treated for 30 days (30 to 60 dpi) with a combined therapeutic strategy with sulfadiazine (S) and pyrimethamine (P). The open field (OF) and elevated plus maze (EPM) tests, widely used to assess hyperactive and impulsive components of attention deficit hyperactivity disorder (ADHD), respectively, were used. Subsequently, the number of cysts present in the brain was evaluated. Hyperactivity was detected in the early (30 dpi) and long-term (60 and 90 dpi) chronic *T. gondii* infection. For example, at 60 dpi infected mice showed increase in walking speed in the OF than non-infected (NI) mice, supporting the presence of hyperactivity. In addition, infected animals remained longer time exploring the open arms of the EPM, when compared to NI, indicative of impulsivity. The S+P treatment reduced the number of cysts in the CNS, when compared to vehicle. However, there was no effect on the hyperactivity observed in the OF, nor on impulsivity in the EPM. **Keywords:** Behavioral tests.ME-49 strain.Hyperactivity

HP-040 - **Expression and functionality of co-inhibitory receptors TIGIT, TIM-3 and LAG-3 in CD4+ T cells from patients with chronic Chagas disease.**

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Chagas disease, caused by *Trypanosoma cruzi*, affects ~7 million people worldwide, mainly in Latin America where it is endemic. Unless treated early after infection, the disease progresses to a chronic form in which some patients develop cardiac or digestive alterations, while others stay asymptomatic. $CD4^+$ T cell response plays critical and diverse roles during infection, and becomes impaired over time with defective cytokine release. This process known as T cell exhaustion is also defined by the upregulation of inhibitory receptors, mainly PD-1 and CTLA-4. In this work, we aimed to explore the expression of the second line of inhibitory receptors TIGIT, TIM-3 and LAG-3 in parasite stimulated $CD4^+$ T cells from patients with different stages of chronic Chagas disease (CCD), and whether their blockade restores cell functionality. Antigen-specific $CD4^+$ T cells were identified by activation induced markers (AIM) assay, using the surface molecules Ox40 and CD25. CCD patients, independently of their clinical stage, showed an increased frequency of $CD4^+TIGIT^+$ T cells. $TIM\text{-}3^+$ $CD4^+$ T cells were more abundant in patients with cardiac manifestations, while $LAG\text{-}3^+$ cells were increased in asymptomatic CCD patients, with higher frequency within the non-activated cells subpopulation upon *T. cruzi* lysate stimulation. Preliminary data showed that TIM-3 blockade tended to increase $IFN\text{-}\gamma^+$ $CD4^+$ T cells in CCD patients with cardiomyopathy. Furthermore, in the same group of subjects, the incubation with an anti-TIGIT blocking antibody led to a greater frequency of $IL\text{-}10^+$ $CD4^+$ T cells compared to the isotype control antibody. Our results highlight the role of the inhibitory receptors TIGIT, TIM-3 and LAG-3 in the modulation of anti-*T. cruzi* $CD4^+$ T cell responses, in relation with the progression of chronic Chagas disease, and paves the way for the development of novel therapeutic strategies. **Supported by:**PIP 2015 **Keywords:** chronic Chagas disease.T-cell exhaustion.inhibitory receptors

HP-041

HOST IMMUNE RESPONSE PARTICIPATES IN MALARIA ACUTE KIDNEY INJURY: ROLE OF CD8+ T CELLS

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Severe malaria is attributed to *Plasmodium falciparum* infection and entail different pathologies caused by direct effects of parasite infection and host immune response. Malaria acute kidney injury (MAKI) is characterized by glomerular and tubular damage. This process is attributed to oxidative stress and obstruction of renal microvasculature caused by aggregates of parasitized red blood cells. However, little is known about the role of immune system in MAKI. The objective of the work was to evaluate the participation of T cells in MAKI pathogenesis. For this, we performed adoptive transfer of splenocytes-derived T cells from C57Bl6 mice infected with *P. berghei* ANKA to healthy acceptor animals. Renal function as well as homing and immune response were assessed. Adoptive transfer induced proteinuria (2-fold) and increased UPCr (protein and creatinine ratio; 2.3-fold). Markers of glomerular injury, creatinine clearance, plasma creatinine and plasma urea, did not change. On the other hand, an increase in gamma GT activation in urine (1.6-fold), a marker of renal tubular damage, was observed. These results indicate that malaria-responsive T cells induce renal tubular damage without glomerular involvement. We observed an increase in homing of malaria-responsive T cells to the spleen, brain and kidneys. An increase in renal proinflammatory cytokines INF□, IL-17 and IL-6 were also observed. When we evaluated the T cells in the kidney, adoptive transfer increased the frequency of renal CD8+ T cells as well as the expression of perforin in the renal cortex, a marker of cytotoxic T cell activity. These results indicate that CD8+ T cells are activated during malaria infection and can migrate to the kidney besides brain and spleen. In the kidney, perforin production has a role in inducing renal tubular damage. This work adds to the literature that MAKI could be also a consequence of an exacerbated host immune response. **Supported by:**Faperj, CNPq e Capes
Keywords: malaria.acute kidney disease.CD8+ T cell, severe disease

PV-001 - **A comparative study demonstrates vertical inheritance and maintenance of arsenic resistance metabolism in eukaryotes**

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Arsenic has been a ubiquitous component, usually in low concentrations, in the environment since primitive Earth. Because arsenic compounds present a certain level of toxicity to all living organisms, it represents a constant environmental pressure on life. Bacteria already have their mechanism of resistance to arsenic thoroughly described. However, these mechanisms are still somewhat obscure for eukaryotes though we know arsenic-resistant lineages in the group. While we currently attribute the origin of the arsenic resistance machinery in eukaryotes to lateral gene transfer (LGT) from bacteria, only a handful of eukaryotes were deeply studied. In this work, we investigate the origin and evolution of resistance to arsenic in eukaryotes using a broader phylogenomic framework. We hypothesize that, since arsenic pressure is constant throughout Earth's history, resistance mechanisms should be present in some way in eukaryotic ancestors. We identified homologs for each of the arsenic resistance genes in eukaryotes and traced their possible origin through phylogenetic reconstructions. We reveal that: i. an important component of arsenic-resistance machinery must have originated before the last common eukaryotic ancestor (LECA); ii. The late events of gene duplication and LGT generated new homologs that, in many cases, replaced the ancestors. Thus, we accepted our hypothesis of ancestral presence, although LGT is an important component in the evolutionary process of these resistance mechanisms. The environment of origin and evolution of the first eukaryotes is still the subject of intense debate. Thus, the selective pressure imposed by arsenic on these organisms is still uncertain. However, as arsenic is constantly present in the environment and we found evidence of ancestral eukaryotic homologs to these metabolisms, we infer that basic resistance machinery must be ancestral and changed throughout the eukaryotic diversification.
Supported by: Fundação de amparo à pesquisa do Estado de São Paulo (FAPESP), processo 2019/22109-0 **Keywords:**Environmental adaptation.Resistance to toxic metalloids.Functional phylogenomics

PV-002 - **Identification of components of a divergent mRNA export pathway in trypanosomes**

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mRNA export is central for gene regulation and expression. In trypanosomes, transcription is polycistronic and all mRNAs are processed by *trans*-splicing, export is mediated by non-canonical mechanisms. We have described several conserved mRNA export pathway components in *Trypanosoma cruzi*, including orthologs of Sub2, a component of the TREX complex, and eIF4AIII, a core component of the exon junction complex (EJC). Few orthologs encoding proteins involved in mRNA export in higher eukaryotes are detectable in trypanosome genomes and examples of mechanistic divergence well known. To uncover additional components of the trypanosome mRNA maturation and export system we undertook an unbiased search for protein interactors of TcSub2 and TceIF4AIII. Significant overlap between TcSub2 and TceIF4AIII interacting protein cohorts suggests that both proteins associate with similar machinery. We confirmed interactions of mRNA export proteins with conserved core components of the EJC and multiple additional complexes together with divergent proteins specific to trypanosomatids. The highly interactive super-interactome uncovered here, capable of supporting RNA processing from splicing through to nuclear export, highlights kinetoplastid-specific and conserved components creating an amalgamation supporting unique mRNA maturation mechanisms in trypanosomes.
Supported by:CNPq 421990/2017 **Keywords:**RNA export.Trypanosomes.divergent proteins

PV-003 - **Phylogenetic reconstruction and evolution of the Rab GTPase gene family in Amoebozoa**

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Rab GTPase is a paralog-rich gene family that controls the maintenance of the eukaryotic cell compartmentalization system. Diverse eukaryotes have varying numbers of Rab paralogs. Currently, little is known about the evolutionary pattern of Rab GTPase in most major eukaryotic 'supergroups'. Here, we present a comprehensive phylogenetic reconstruction of the Rab GTPase gene family in the eukaryotic 'supergroup' Amoebozoa, a diverse lineage represented by unicellular and multicellular organisms. We demonstrate that Amoebozoa conserved 20 of the 23 ancestral Rab GTPases predicted to be present in the last eukaryotic common ancestor and massively expanded several 'novel' in-paralogs. Due to these 'novel' in-paralogs, the Rab family composition dramatically varies between the members of Amoebozoa; as a consequence, 'super group'-based studies may significantly change our current understanding of the evolution and diversity of this gene family. The high diversity of the Rab GTPase gene family in Amoebozoa makes this 'supergroup' a key lineage to study and advance our knowledge of the evolution of Rab in Eukaryotes. **Supported by:**FAPESP: 2017/19388-0, 2019/22692-8 e 2016/14317-4 **Keywords:**Phylogenomics.Eukaryotic evolution.Rab therapeutic intervention

PV-004 - **Characterization of a Fatty-Acid Binding Protein-like in *Leishmania amazonensis***

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Leishmania parasites are the causative agents of a group of diseases collectively known as leishmaniases. These neglected tropical diseases are endemic in Africa, Southeast Asia, and South America, where more than 1 billion people are at risk of infection. There are no vaccines and therapy remains limited due to drugs toxicity and resistance. During their life cycle, *Leishmania* alternate 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*. At first, we designed the primers and optimized the PCR conditions to amplify the open reading frame of *L. amazonensis LeiFABP*. At the moment, we are generating the constructs for expression of LeiFABP fused to GFP using the *Leishmania* expression plasmids pXG-GFP+ and pXG-GFP2+. With this, we will confirm the subcellular localization of LeiFABP in *L. amazonensis* and characterize the overexpressing parasites regarding replication and virulence. LeiFABP characterization may help elucidate the distinctive features of *Leishmania*' FAs metabolism and trafficking, indicating new targets for the development of better therapeutic strategies against leishmaniases.
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Keywords: Leishmaniasis.lipid metabolism.neglected tropical diseases

PV-005 - **Could inositol pyrophosphates have been influencing parasitism within kinetoplastids?**

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Inositol pyrophosphates (e.g.: IP_7 and IP_8) are highly energetic metabolites involved in a wide range of cellular processes. IP_7 and IP_8 are synthesized through complementary pathways that involve IP6K and PP-IP5K kinases, respectively. Trypanosomatids parasites (e.g.: *Trypanosoma cruzi* and *Leishmania* spp.) have orthologous genes for IP6K, but the genes encoding to PP-IP5K are apparently absent. Curiously, *Bodo saltans* (a free-living kinetoplastid organism) own a PP-IP5K homolog with 42% identity relative to human PP-IP5K. The goal of this study is to investigate whether the absence of PP-IP5K (and consequently IP_8) is mutually exclusive relative to parasitism within kinetoplastids. Thereunto, we carried out evolutionary analyzes using IP6K and PP-IP5K kinases to confirm the alleged loss of PP-IP5K. Using deep analyzes with HMMER, MUSCLE, TRIMAL, and PHYML, we reconstructed phylogenetic trees and get robust evidence that confirms the absence of PP-IP5K in all trypanosomatids but *Paratrypanosoma confusum*. Predictions of the tertiary structures pointing that the catalytic domain of *P. confusum* PP-IP5K, although it has 44% identity to human PP-IP5K, is naturally unstructured, which puts its function in suspicion. Our findings, although preliminary, suggest that the transition from a free-living to a parasitic lifestyle has resulted in the loss of PP-IP5K. Our next steps include performing PP-IP5K knock-in (from *Bodo saltans*) in *T. cruzi* using CRISPR/Cas9 system. We intend to perform a phenotypic characterization of the generated KI lineage, in addition to conducting infection assays. Also, we intend to perform knock-out (KO) of PP-IP5K in Vero cells (used as a host cell) to evaluate infection alterations. This work will clarify if the PP-IPs have any relation with the parasitism developed within the kinetoplastids, contributing to unveil new routes for the developing antitrypanosomal therapies. **Supported by:**CAPES, FAPESP (2019/10753-2; 2020/10277-3) **Keywords:**inositol pyrophosphates.knock-in.parasitism

PV-006 - **Effect of the calcium binding protein TcCAL1 on the host-cell invasion by Trypanosoma cruzi**

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Calcium (Ca^{2+}) plays crucial role in the life cycle of *T. cruzi*. The parasite genome encodes for several Ca^{2+}-binding proteins, although most of them remain uncharacterized. TcCAL1 is a hypothetical Ca^{2+}-binding protein with EF-Hand motifs identified through proteomic analysis of *T. cruzi*. Our work aims to unravel the role of TcCAL1 in the *T. cruzi* life cycle. Previously, we found that the overexpression of TcCAL1 decreased the metacyclogenesis rates of epimastigotes *in vitro*. Now, we evaluated the virulence of TcCAL1-overexpressing parasites in mammalian cell invasion assays. To study this, the gene encoding tccal1 was fussed to a 6x histidine tag and cloned into the pTREX expression vector. Epimastigotes from strain Y were transfected with the plasmid pTREX/TcCAL1x6His or the empty vector pTREX (controls). After selection, recombinant epimastigotes were differentiated into metacyclic trypomastigotes in blood-agar/LIT medium and then incubated for 24h with Vero cells using a 300:1 parasite:host cell ratio. At 48h post infection, samples were Giemsa-stained to calculate the percentage of infected cells and the number of intracellular amastigotes by counting three hundred Vero cells per sample in randomly selected fields; results were compared by using the Student´s t-test. Preliminary data showed that the percentage of infection significantly increased in host cells incubated with TcCAL1x6His-overexpressing parasites in comparison to controls (41.2% versus 18.9%, respectively; P = 0.0092), as well as the number of internalized amastigotes (1398 versus 926 parasites/100 cells, respectively; P = 0.024). Based on this finding, we hypothesize that high levels of TcCAL1 protein disrupt the intracellular Ca^{2+} concentration interfering with *T. cruzi* differentiation and increasing host cell invasion. Future experiments will be focused on the validation of the effects of TcCAL1 on cell invasion and the experimental verification of Ca^{2+} binding by TcCAL1. **Supported by:**PICT 2016 NUMBER 1028 INGEBI FOUNDATION **Keywords:**Trypanosoma cruzi., Ca2+-binding protein.host-cell invasion

PV-007 – **Investigating the Kennedy Pathway:phosphatidylcholine and phosphatidylethanolamine synthesis in *Trypanosoma cruzi***

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The Kennedy pathway is the main biosynthetic route for ph and phosphatidylethanolamine (PE) formation. The importance of P sitic protozoa has been well demonstrated. Disruption of PC um *falciparum* and *Leishmania donovani;* disruption of PE h nosoma *brucei*. However, information is severely lackin etabolism in *Trypanosoma cruzi*.
Although bioinformatic analysis of the T ngle bifunctional choline and ethanolamine kinase at the s investigation revealed two functional kinases. These pu pressed in *Escherichia coli*, purified and characterised. Kine 35.190 (*Tc*E/CK), is bifunctional, with a higher affinity for ontrastingly, *Tc*CLB.487739.20 (*Tc*C/EK), although also choline than ethanolamine.
*Tc*E/CK ' he subcellular localisation of *Tc*E/CK was determined to n alleles of *Tc*E/CK was only possible in the presence of an e g essentiality. At present, metabolomic phenotyping is underway for lines as well as in vivo characterisation of *Tc*C/EK.
The nes for compensatory PE/ PC biosynthesis suggests *T. cruzi*, like *T. brucei*, relies the Kennedy pathway. This will allow drug selectivity, as humans have additional, alterna ates for PE/PC biosynthesis. In conclusion, the enzymes of the Kennedy pathway in *T. cruzi* ar appealing drug targets with a fundamental role in parasite survival and warrant further investigation. **Supported by:**EPSRC (UKRI) and University of St. Andrews **Keywords:**Metabolism.Phospholipid.Trypanosoma cruzi

PV-008 - **An unconventional RNA-binding protein defining the mRNA fate of the major variant surface antigen.**

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The variant surface glycoprotein (VSG) enables salivarian trypanosomes to evade host's adaptive immunity via antigenic variation. VSG mRNA comprises 10% of total cell protein and the high stability of VSG mRNA is essential for VSG expression, and so trypanosome survival. To determine how VSG mRNA stability is maintained, we used mRNA affinity purification to identify all its associated proteins. CFB2 (Cyclin F-box protein 2), an unconventional RBP, was specifically enriched with VSG mRNA. We demonstrate that CFB2 is essential for VSG mRNA stability, describe cis acting elements within the VSG 3'-untranslated region that regulate the interaction, identify trans-acting factors that are present in the VSG messenger ribonucleoprotein particle, and mechanistically explain how CFB2 stabilizes the mRNA of this key pathogenicity factor.
CFB2 has an N-terminal cyclin-F-box domain. F-box proteins are found in SCF complexes (SKP1-Cullin-F-Box), which are E3 ligase components of the ubiquitination machinery. We have demonstrated that the interaction between CFB2 and SKP1 is involved in autoregulation of CFB2 abundance. Ongoing biochemical studies aim to scrutinize the novel RNA binding domain and find out whether there is a link between SCF-complex formation and nucleic acid binding activity. **Supported by:**H2020 European Research Council (649019) /core funding from the University of Heidelberg/ **Keywords:**Trypanosoma brucei.VSG.RNA binding protein

PV-009 - **How much does it cost to build a trypanosome? Estimating the amount of ATP necessary for maintaining and duplicating a bloodstream-form Trypanosoma brucei cell**

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ATP is the major energy currency of cells since its hydrolysis generates free energy which is used to power biological processes. ATP hydrolysis is required for synthesis, transport and polymerization of monomers for macromolecules as well as for the assembly of the latter into cellular structures. ATP is also necessary for cellular processes not directly related to the synthesis of biomass, such as maintenance of membrane potential and cellular shape. *Trypanosoma brucei* is a unicellular flagellated parasite with a complex life cycle occurring between an insect vector and a mammalian host. Glucose, abundantly available in the bloodstream of the mammalian host, is used as the main energy source by the bloodstream form (BSF) of this parasite. BSF *T. brucei* has been shown to use very little or no carbon from glucose for the synthesis of its biomass, but to depend on the extracellular availability of other essential nutrients as carbon sources for the biosynthesis of precursors. Approximately 97% of the glucose consumed by the BSF is directed to ATP synthesis. Here, we made a detailed estimation of the energy budget during the BSF cell cycle, with the energy costs of the cell's two main commitments: to stay alive and to duplicate. As glycolysis is the only source of the produced ATP, we calculated that a single parasite produces $5.9x10^{11}$ molecules of ATP/cell cycle. We found that biomass production accounts for 43% of the total energy budget, with translation being the most expensive process. Interestingly, the budget for flagellar motility, VSG recycling, maintenance of transmembrane ion and proton gradients and ATP-dependent transport processes accounts for 32% of the total ATP. Finally, there is still 25% available in the energy budget that is being used for other cellular processes of which the relative costs remain to be elucidated. These data put a new perspective to the assumptions about the relative weight of cellular processes BSF undergo during its cell cycle. **Supported by:**Fundação de Amparo à Pesquisa do Estado de São Paulo **Keywords:**Trypanosoma brucei.Metabolism.ATP

PV-010 - **Biological evaluation of (E)-N'-(thiophen-2-yl methylene)benzohydrazide as an antileishmanial agent**

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Leishmaniasis, caused by protozoa *Leishmania sp*, is a neglected tropical disease that affects between 12 and 15 million people in the world. Currently, the drugs available are limited, toxic and with low effectiveness. Thus, given the difficulties involved in its treatment, the search for new efficient and less toxic drugs is a priority in the medical field. This study evaluated the in vitro activity of (E)-N'-(thiophen-2-yl methylene)benzohydrazide (BH6), a new n-acylhydrazone, against promastigotes of *L. amazonensis*. The antiproliferative activity was determined by XTT method and IC_{50} and IC_{90} were calculated by linear regression. Further, ultrastructural changes were observed by transmission electron microscopy (JEOL JM1400) after 72 h of treatment. Fluorimetric analyses were performed in fluorescence microplate reader (VICTOR X3, PerkinElmer) to investigate ROS generation using H2DCFDA and lipid inclusions using Nile Red and by flow cytometry (BD FACSCalibur) to evaluate mitochondrial membrane potential with Rhodamine 123 and membrane integrity with propidium iodide (PI). The results showed an antiproliferative activity, with an IC_{50} and IC_{90} of 7.18 and 19.66 µM, respectively. Promastigotes treated with BH6 revealed important ultrastructural changes, such as mitochondrial swelling, concentric membranes inside the mitochondria and cytoplasm, presence of autophagosomes and lipidic inclusions. Moreover, BH6 induced an increase in intracellular ROS levels after 24 h treatment, and a decrease in mitochondrial membrane potential. There was no increase in PI-positive cells, indicating that the plasma membrane appears to be intact. In addition, there was an augmentation in lipid inclusions, corroborating with TEM observations. These findings suggest that BH6 induced oxidative damages, probably leading to cell death. Some studies are still needed to define the mechanism of action of this compound; however it has shown a potential activity against *L. amazonensis*. **Supported by:**Capes, CNPq, FINEP and Pronex/Fundação, COMCAP-UEM **Keywords:**antiproliferative activity.Leishmania amazonensis.oxidative stress

PV-011 - **The Fantastic Four: identification of four cruzipains subtypes in *T. cruzi***

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Cruzipain is the main cysteine protease of *Trypanosoma cruzi,* associated with metacyclogenesis, host cell infection, and modulation of the immune response. Initial works from the ’90s describe dozens of cruzipain gene copies in *the T. cruzi* genome. However, genome data obtained with Sanger or Second Generation Sequencing did not allow determining the number of cruzipain copies. In the past few years, *T. cruzi* genomes were sequenced with Third Generation Sequencing. These new technologies generate longer reads, having better performance in repetitive regions than the previous ones. Thus, we investigated the genomic localization, the number of copies, and the phylogenetic relationship of cruzipain genes in three *T. cruzi* genomes sequenced with PacBio and Nanopore. We searched for cruzipain sequences in Dm28c (TcI), YC6 (TcII), and CL Brener (TcVI) with Blast. The gene sequences were aligned with Clustal Omega, and phylogenetic trees were constructed with MEGA-X. We also evaluated the expression of cruzipains copies with RNA-Seq data available from the CL Brener strain. The new subtypes identified by us had their 3D structure predicted by homology modeling. Based on the phylogenetic reconstructions and analysis of the sequences, we subdivided the cruzipains into two Families and four subtypes. Family I comprises czp1, the first described in the literature and targeted in drug design efforts for Chagas disease. Family II comprises czp2, which also has been previously described, and the novel czp3 and czp4. The four of them have significant differences in the active site residues, which likely impact their substrate specificity and interactions with small molecules. Family I sequences are mostly expressed in epimastigotes, while the Family II ones in the mammalian forms. Our results suggest the importance of the biological and biochemical characterization of all cruzipain subtypes to better understand *T. cruzi* biology and improve drug design for Chagas disease.
Supported by:CAPES, CNPq, FAPEMIG, FAPERJ, and FAPESP **Keywords:**cruzipain,Trypanosoma cruzi, cysteine protease

PV-012 - **Investigation of kDNA minicircles copy number in *Trypanosoma cruzi* genetic groups by duplex qPCR**

NAVES, L.L.; DA SILVA, R.B.; DOS SANTOS, M.C.; SILVA, E.L.; GIRALDO, L.E.R.; PEDROSA, A.L..
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Chagas disease is caused by *Trypanosoma cruzi*, a protozoan belonging to the order Kinetoplastida. Organisms of this order possess DNA in the nucleus and in a single mitochondrion whose DNA is organized the kinetoplast, an organelle composed of minicircles and maxicircles of kinetoplast DNA (kDNA). T. cruzi population can be divided in six genetic groups (TcI - TcVI) and the contribution DNA in present in kinetoplast and nucleus for total DNA the parasites remain elusive. The main aim of this work is to investigate kDNA minicircles copy number in strains and clones of different T. cruzi genetic groups. Genetic characterization by PCR-RFLP of nine *T. cruzi* strains/clones associated them to TcI (n=2), TcII (n=3), TcIII (n=1), TcV (n=1), and TcVI (n=2) genetic groups. In duplex qPCR, the single copy gene DHFRTS (dihydrofolate reductase - thymidylate synthase) was used as a reference gene for quantification of kDNA minicircles. No significant difference among normalized Cts was observed in four different DNA concentrations (0.005 to 5 ng of DNA) tested. We used the Pfaffl method to correct relative efficiencies. Lin’s coefficient was used to predict the number of parasites according to the estimated values of normalized Cts determined by duplex qPCR. *T. cruzi* JG strain (TcII) was the one that presented the highest kDNA minicircles copy number (1284 copies per copy of DHFRTS) and the CL Brener clone (TcVI) had the lowest kDNA minicircles copy number (7.49 copies per copy of DHFRTS). These results revealed a great variation in kDNA minicircles copy number in *T. cruzi* strains analyzed. These differences can be due to real variations in minicircles copy number, but we cannot exclude the possibility that polymorphisms in the annealing sites of primers and/or probes may have influenced quantifications. Further studies using different primers or probes can reveal the complete extension of differences of kDNA in the genetic groups of *T. cruzi*. **Supported by:**CAPES,CNPQ, FAPEMIG **Keywords:**trypanosoma cruzi.kDNA.DNA content

PV-013 - **Role of histone-fold domain containing Oocyst Rupture Proteins during oocyst and sporozoite stages**

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Sporozoites are produced in the mosquito inside the oocyst roughly 12 days after the uptake of an infected blood meal. Oocyst rupture is required for the release of the sporozoites, which next travel to the salivary glands where they will be transmitted to the new host. In *Plasmodium* two proteins containing histone-fold domains (HFD), which are usually found in DNA binding proteins, have essential roles in this process. We named these proteins ORPs (Oocyst Rupture Proteins) as mutant parasites lacking either one of the *orps* are unable to exit from the oocyst. Motile sporozoites develop normally but they remain trapped inside the intact oocyst capsule, leading to a complete block in transmission to mice. ORP1 is expressed in the cytoplasm of all *Plasmodium* stages and in oocysts it localizes to the oocyst wall. ORP2 is detected only in the cytoplasm of young oocysts and at the oocyst wall after sporozoites are formed. The HFDs of the two ORPs are directly implicated in the mechanism of oocyst rupture, possibly through the formation of a dimer. ORP1 and ORP2 have HFDs similar to subunits of the NF-Y transcription factor of higher eukaryotes but the parasite proteins are much bigger. Our recent data on progressive deletions of ORP2 show that other portions of ORP2 may play a role in the localization of the protein at the mature oocyst wall thus promoting the interaction of the HFDs of the two proteins and capsule rupture. Furthermore, mutant sporozoites lacking *orp1* show a defect in gliding motility and invasion of liver cells and ORP1 is expressed in early liver stages suggesting a function of this protein also in these stages of the parasite. Our data show that *Plasmodium* exploited the DNA binding HFD for a divergent function in the unique process of oocyst wall rupture. ORPs, or their specific domains, could be a possible target for anti-malarial strategies development to stop malaria transmission to the vertebrate host.
Keywords:anófeles,malária,oocyst

PV-014 - **Could the roles played by inositol pyrophosphates (PP-IPs) in trypanosomatids be related to DNA metabolism and virulence?**

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In model eukaryotes, inositol pyrophosphates (PP-IPs) are involved in a wide range of processes, such as telomere homeostasis and homologous recombination. However, the mechanism of action of these metabolites is not fully understood. The PP-IPs (IP_7 and IP_8) are synthesized by pathways involving the participation of IP6K and PP-IP5K kinases, respectively. *Trypanosoma cruzi* and *Leishmania* spp., etiological agents of neglected diseases, apparently do not present homologs for PP-IP5K. Also, DNA metabolism in these organisms presents peculiarities that seem to be exclusive to this family. The goal of this study is to elucidate the roles of PP-IPs in DNA metabolism, and parasitism developed by trypanosomatids. For this, we first carried out in silico analysis. We confirmed the absence of PP-IP5K in all trypanosomatids but *Paratrypanosoma confusum*. Predictions of the tertiary structures pointing that the catalytic domain of *P. confusum* PP-IP5K, although it has 44% identity to human PP-IP5K, is naturally unstructured. Curiously, *Bodo saltans* (a free-living kinetoplastid) own a PP-IP5K homolog with 42% identity relative to human PP-IP5K. This finding suggests that the transition from a free-living to a parasitic lifestyle has resulted in the loss of PP-IP5K, which makes us wonder if the absence of PP-IP5K (and consequently IP_8) is mutually exclusive relative to parasitism within kinetoplastids. To investigate that in deep, we are performing knock-in (KI) of PP-IP5K from *B. saltans* into *T. cruzi* and *L. braziliensis*. Next, we intend to evaluate the virulence of the KI strains generated. We are also performing assays to deplete the IP6K gene in *T. cruzi* and *L. braziliensis* to investigate the participation of IP_7 in DNA repair, and infection capacity. Finally, using a groundbreaking approach, we intend to track the peptides pyrophosphorylated by IP7. The participation of PP-IPs in these pathways could provide new routes for developing antiparasitic therapies. **Supported by:**São Paulo Research Foundation (FAPESP) 2019/10753-2; 2020/10277-3 **Keywords:**Inositol pyrophosphates,DNA repair,virulence

PV-015 - **Characterization of *Blastocystis* subtypes isolated Minas Gerais State, Brazil**

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Blastocystis is an intestinal protozoan that has gained relevance in recent years owing to its possible role in the pathogenicity of the human hosts. Although underestimated, the prevalence of *Blastocystis* spp. in human hosts ranges from 17.8% to 86.63% in Brazil. It is known that humans are mainly infected by ST1–ST4 and rarely by ST5–ST9. In Brazil, the subtypes ST1 - ST4 and ST6 - ST8 have already been demonstrated. However, the molecular epidemiological profile of *Blastocystis* has not been established in several cities and regions of Brazil. The main aim of this study is to genetically characterize the isolates of *Blastocystis* species obtained from human and animal fecal samples previously positive for the parasite and investigate the presence of Blastocystis in the water supply of a city in the interior of Minas Gerais. SSU rDNA gene PCR amplicons were generated from 21 *Blastocystis* isolates obtained from humans, one bovine isolate, four pig isolates and from 18 water samples. Amplicon sizes obtained varied (0.9 to 1.3 kb) from the expected (1.1 kb) in 18/21 isolates from humans and 17/18 water isolates. PCR-RFLP demonstrated the presence of the subtypes ST1 (53.3%), ST3 (40.0%) and ST2 (6.7%) for human isolates; ST10 (100%) for bovine isolate; and ST5 (50.0%), ST1 (25%), and ST3 (25%) for pig isolates. Sequencing of PCR products showed 98–99% identity to the *Blastocystis* sequences deposited in Genbank, except for sequences from water samples that showed the highest identity with algae rDNA. **Conclusion**: Main *Blastocystis* subtypes circulating in the Triângulo Mineiro, Brazil, are ST1-ST3, ST5, and ST10. *Blastocystis* ST1 and ST3 found in humans and pigs reinforce the zoonotic transmission potential of the parasite. *Blastocystis* specific primers should be designed to investigate the parasite in environmental samples.
Supported by:CAPES **Keywords:**Blastocystis.Subtypes.Genetic characterization

PV-016 - **Chemical genetic approaches to study the role of protein kinases in *Leishmania* cell cycle regulation.**

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Cell division is a core biological process during the development of both multicellular and unicellular organisms. It is a conserved process throughout eukaryotes, which has diverse evolutionary roots, resulting in a unique repertoire of protein component in Trypanosomatids, including *Leishmania*. Some of these components has been identified in *Leishmania*, but the extension of its repertoire and their role in the coordination of the cell cycle remains unclear. Here, we used genetic and chemical approaches to explore the role of some essential protein kinases in cell cycle progression. We used CRISPR-Cas9 to perform precision editing of the *L. mexicana* genome to generate analogue sensitive mutants suitable for chemical genetic inhibition. For the kinetochore protein kinase KKT2, CRK9 and CMGCa replacement of the bulky gatekeeper methionine residue with a glycine in the ATP-binding site makes the enzymes sensitive to the bulky inhibitor 1NM-PP1. For the CLK1 and CLK2 replacement of a cysteine near to the ATP-binding domain prevents binding of the inhibitor AB1, validating the specificity of this compound against CLK1/CLK2. The specific inhibition of CLK1, CLK2, KKT2 caused a cell cycle arrest in G2/M stage of the promastigote. A further investigation, by fluorescence microscopy labelling the mitotic spindle, revealed that KKT2 inhibition is followed by a significant accumulation of cells in early mitosis, where mitotic spindle coordination in the nucleus failed. Moreover, it was observed that CMGCa inhibition also impaired chromosome segregation, but the cell body development reaches a more advanced stage, suggesting CMGCa activity is required later in mitosis than KKT2. In addition, CLK1/CLK2 inhibition doesn't affect the coordination of the mitotic spindle, but it blocks cell cycle progression in cytokinesis. These studies bring new insights into the essential biological process of cell division in *Leishmania* and provide a source of new potential therapeutic targets. **Supported by:**GCRF **Keywords:**L. mexicana, Chemical genetic approaches,Cell Cycle

PV-017 - **Could the kinase IP6K influence telomere dynamics in *Leishmania braziliensis*? Preliminary analyses**

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In model eukaryotes, inositol pyrophosphates (PP-IPs) are involved in a wide range of cellular processes, such as telomere length regulation and homologous recombination. However, the target proteins of PP-IPs, as well as their mechanism of action, are still not well established. The main PP-IPs involved in these processes are the so-called IP_7 and IP_8, which are synthesized by pathways involving the kinases IP6K and PP-IP5K, respectively. Trypanosomatid parasites, such as *Leishmania* spp., have homologs genes for IP6K but do not have homologs for PP-IP5K, making them excellent models study of IP_7. This work aims to generate a lineage KO for IP6K ($IP6K^{-/-}$ or $IP6K^{-/+}$) in *Leishmania braziliensis*, using the CRISPR/Cas9 approach. Then, we intend to evaluate the telomere homeostasis relative to wild type (WT) after shortening the telomeres of IP6K KO lineage with H_2O_2. Currently, we are carrying out the transfections using an *L. braziliensis* lineage expressing T7-Cas9 and two DNA donors containing a different resistance marker each (puromycin and blasticidin). After performing clone selection through serial dilutions, we will confirm the depletion of IP6K (generating lineages $IP6K^{-/-}$ or $IP6K^{-/+}$) by PCRs and qPCR. Episomal IP6K add-back lineages will be generated to demonstrate the specificity of the assay and eliminate bias due to off-targets. Next, using the IP6K KO lineages ($IP6K^{-/-}$ or $IP6K^{-/+}$) and controls (WT and add-back), we will perform growth curves, FACS analysis, and we will standardize the STEX (single telomere extension) assay to evaluate the telomere dynamics. It is worth mentioning that this work will help us to answers questions of fundamental importance, such as: Could the *Leishmania* telomeres be used as a new route for the development of antiparasitic drugs? The kinase IP6K (or the inositol pyrophosphate IP_7) could contribute to that? This project will be of great relevance to answer these questions. **Supported by:**FAPESP - 2020/16481-1 **Keywords:**CRISPR/Cas9, inositol pyrophosphates, telomere dynamics

PV-018 - **Genomic comparison of *Trypanosoma cruzi* to trypanosomes of the clade *T. cruzi* isolated from bats**

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Bat trypanosomes have been implicated in the evolutionary history of *T. cruzi* clade. It has been suggested that *T. cruzi* and *T. rangeli* evolved from a lineage of bat trypanosome that later adapted to terrestrial mammals. Here we study the karyotype organization and syntenic associations between the species of clade *T. cruzi*, especially those infecting bats (*T.cruzi bat*- Tc bat, *T. cruzi marinkellei*- Tcm and *T. dionisii*- Tdio). Tc bat and Tcm karyotypes are quite similar in number and size of chromosomal bands to the *T. cruzi* lineage TcI. While, the number and size of bands of Tdio were lower than those of *T. cruzi*. Chromosome synteny across bat trypanosomes was demonstrated by chromoblot hybridization with specific markers of syntenic blocks conserved in *T. cruzi* lineages. We performed comparative genomic hybridization (aCGH) to identify chromosome regions harboring copy number variations (CNV) in bat trypanosomes using the genome of *T. cruzi*- CL Brener as a reference. We found 410 CNVs distributed as follows: 295 in Tcbat, 93 in Tcm and 104 in Tdio. Most commonly observed aberrations were deletions and duplications of small chromosomal segments (<50kb) which may be due to homologous recombination. Alterations comprising the entire length of chromosome have also been identified, featuring a chromosomal aneuploidy event. Our data indicates that these changes have occurred in chromosome counterparts in Tcm and Tcbat. Aneuploidy events can be explained by non-disjunction of counterparts in mitosis or by overreplication of one homologue and/or under-replication events. Other chromosomal aneuploidy events have been identified in the homologues of in Tcm, Tcbat and Tdio. We note that changes in isolates Tcm, Tcbat and Tdio were flanked by members of the multigene families suggesting that these genes play an important role in recombination events. **Supported by:** CNPq -Número de processo:134397/2015-0 **Keywords:**Trypanosoma cruzi, karyotype, Genomic comparison

PV-019 - **The knockout of a noncoding RNA differentially expressed in Leishmania braziliensis emerges from the 3'UTR of the FtsX-like gene, regulates its expression and promotes phenotypical changes**

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Leishmania braziliensis (Lbr) is the main etiological agent of cutaneous leishmaniasis in Brazil. Such parasites have an unusual mode of gene expression control and mRNAs processing, compared with other eukaryotes. We have previously shown the presence of stage-preferentially expressed noncoding RNAs (ncRNA) during the life cycle of *Lbr*. Herein, we selected some of these differentially expressed ncRNAs to evaluate putative functional roles and phenotypical changes, based on the individual knockout (KO) in promastigotes expressing the CRISPR/Cas9 system. Four ncRNAs were successfully knocked out in *Lbr* and here, we highlight the 20.1_ncRNA97 KO, which showed a remarkable decline in the log phase progression of promastigotes, lower metacyclogenesis rate and increased sensitivity response to nutritional and oxidative stresses in promastigote and axenic amastigote stages, respectively. According to three different *in silico* ncRNA predictors, the 20.1_ncRNA97 was identified as a ncRNA. A RNAseq analysis indicates that such ncRNA might be part of the 3'UTR of the upstream gene LBRM2903_200026000 (FtsX-like protein), but by circular RT-PCR assay and sequencing of the 20.1_ncRNA97 transcript, a 147 bp-long polyadenylated transcript was confirmed. In fact, the expression of the upstream coding gene was negatively affected in the KO parasites. Little is known about FtsX protein in *Leishmania*, but in other organisms it is reported as a transmembrane protein, vital to the final step of the cell division. Consequently, KO parasites with lower FtsX gene expression had their cell cycle recovery slower after the hydroxyurea synchronisation, suggesting the relevance of 20.1_ncRNA97 as regulator of FtsX gene expression, either as a cis- or a trans-element. Northern blotting, comparative transcriptomics and pull-down assays will give us general and robust information to unveil the real function of this ncRNA for the parasite development. **Supported by:**Capes, CNpq, FAPESP (18/14398-0; 2020/00088-9)
Keywords: Leishmania, ncRNAs, Gene expression

PV-020 - **What would be the target proteins of inositol pyrophosphates (PP-IPs) in the human pathogen Leishmania braziliensis? A preliminary approach**

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In model eukaryotes, inositol pyrophosphates (PP-IPs) – mainly IP_7 and IP_8 – are involved in a wide range of cellular processes. However, target proteins of PP-IPs, as well as their mechanisms of action, are still poorly understood. IP_7 and IP_8 are synthesized through complementary pathways that require the kinases IP6K and PP-IP5K, respectively. Trypanosomatids, single-celled eukaryotes parasites (e.g., *Leishmania* spp.), have an ortholog of IP6K, but lack orthologs of PP-IP5K, meaning they are unable to synthesize IP_8. This feature makes trypanosomatids good models to study IP_7. Thus, the goal of this study is to track and identify the main target proteins of IP_7. To pursue this goal, we performed the cloning of recombinant IP6K (as well as its catalytic domain), and IP5K (which will be used as control) using the pET28 system. Preliminary results showed that these kinases are expressed after induction of 1mM IPTG. We intend to purify these proteins and use them to synthesize *in vitro* IP_7 labeled with γ-(Propargil)-imido on the β-phosphate moiety (IP_7-labeled). As a control, IP5K recombinant will be used to synthesize IP_6-labeled (which is a non-pyrophosphate). This approach will allow us to selectively conjugate biotin, via a click chemistry reaction, to *L. braziliensis* proteins that have received the β-phosphate from IP_7-labeled. We will then isolate biotin-labeled proteins using strepdavidin immunoprecipitation and identify them using mass spectrometry. Given pyrophosphorylation by PP-IPs is not enzymatic and still a poorly understood mechanism, our work may uncover new routes for drug development and, therefore, the effective treatment of leishmaniasis. **Supported by:**FAPESP 2020/16465-6 **Keywords:**Inositol pyrophosphates, Recombinant proteins, Mass spectometry

PV-021 - **Attempts of generation of Leishmania infantum deficient in ascorbate peroxidase using CRISPR/Cas9**

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Ascorbate peroxidase (APX) is a redox enzyme of the trypanothione pathway that converts hydrogen peroxide into water molecules regulating the oxidative stress in *Leishmania* and avoiding cell damage. The aim of this study was to delete the APX gene in *L. infantum* to investigate the role of this enzyme in this parasite. The APX (LINF_340005600) knockout attempt was performed using CRISPR/Cas9 approach according to the protocol described by Beneke et al. (2017). The pTB007 plasmid, which has hygromycin as a resistance marker, was used to express *Sp*Cas9 and T7RNAP. *L. infantum* RPV (MHOM/BR/2002/LPC-RPV) promastigotes parasites carrying this plasmid and successfully expressing Cas9 were transfected with donor DNAs and guide RNAs chosen using the LeishGEdit tool. The selection of *Leishmania* clones was done by plating the parasites in semi-solid M-199 medium containing the selective drugs. Deletion assessment was performed by PCR to evaluate the presence of the APX coding sequence, and also the presence of the resistance marker sequences. The results showed the presence of a 1077 bp fragment which corresponds to neomycin resistance marker, indicating the correct integration of donor DNA in the transfected parasites. However, PCR results also demonstrated the amplification of a 912 bp fragment in the mutant parasites, showing that copies of the APX gene were retained by aneuploidy or gene amplification. RT-qPCR and Western blot assays revealed that APX transcript and protein levels in APX *L. infantum* mutant clones were similar to wild-type parasites. The growth of promastigote forms of WT and APX mutant clones and the susceptibility of these parasites to trivalent antimony were also evaluated, but no significant difference was observed between the wild-type line and the mutants. The unsuccessful attempts using CRISPR/Cas9 method to delete APX suggest that this gene is essential in *L. infantum*. **Supported by:**FAPEMIG (FAPEMIG - CBB-PPM and APQ-02816-21); CNPq (CNPq 304158/2019-4); CAPES (001) and Programa Inova FIOCRUZ (VPPCB-007-FIO-18-2-94) **Keywords:**Leishmania infantum, ascorbate peroxidase, CRISPR/Cas9

PV-022 - **Investigation of the role of the ABC proteins (subfamily F) in paromomycin resistance in *Leishmania amazonensis***

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Leishmaniasis is a parasitic disease with wide geographical distribution in tropical and subtropical areas, including Brazil. There are no vaccines available to control the disease and chemotherapy is restricted to some toxic drugs. Although not approved for the treatment of leishmaniasis in Brazil, paromomycin has been used in the treatment of visceral leishmaniasis in the South from Asia, with good clinical efficacy. Studies in other organisms have shown that the proteins of the subfamily ABC-F (ATP Binding Cassette) affect the susceptibility to paromomycin, such as, for example, the elongation factor 3 (EF-3) involved in the translation process of *Saccharomyces cerevisiae*. The search for homologues of these proteins in the databases of the *Leishmania amazonensis* genome identified three genes in the genome this species: *ABCF1*, *ABCF2* and *ABCF3*, as well as in other *Leishmania* species. In this work, the possible involvement of these proteins in susceptibility and resistance to paromomycin was investigated, using molecular genetic techniques such as overexpression and gene inactivation of *ABCFs* genes of *L. amazonensis*. Once the respective transgenic lines were selected, it was found that the promastigote forms of the transgenic lines did not have their *in vitro* growth altered significantly in relation to the wild-type. The overexpressing line of the *ABCF1* gene showed an EC_{50} value of about 1.7 times greater than the wild-type, indicating that overexpression of this gene confers resistance to paromomycin. It was only possible to generate null mutants for the *ABCF2* gene, indicating that the *ABCF1* and *ABCF3* genes are essential for the parasite in the promastigote stage.The null mutants for the *ABCF2* gene showed an EC_{50} value that corresponded to half the EC_{50} value of the wild-type strain, suggesting a possible involvement in the susceptibility to paromomycin due to the *ABCF2* protein. **Supported by:**Fundo de Apoio ao Ensino à Pesquisa e à Extensão (FAEPEX) no processo nº2608/19 **Keywords:**Leishmania, ABC-F.paromomycin

PV-023 - **Identification of aneuploidy events in *Trypanosoma cruzi* by FISH assay**

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T. cruzi shows remarkable genetic variability traits which may play an important role in its adaptative response in the hosts. Even though *T. cruzi* is considered a diploid organism, whole genome sequencing analysis has been shown the presence of aneuploidy in this parasite which may be an important factor in generating genetic variability. We investigated the presence of aneuploidy in *T.* cruzi by fluorescence *in situ* hybridization (FISH) that allows the evaluation of ploidy at the individual cell level. We used single-copy genes (H49 and JL8) as specific-markers of chromosome TcChr39 of CL Brener. They encode repetitive antigens carrying a large tandem array of amino acid motifs. The central region of genes H49 and JL8 comprises an uninterrupted large array of tandem repeats which is a good target to DNA hybridization. The repeats were labeled with Digoxigenin-11-dUTP, cleaved with Dnase I into ≤ 100 bp fragments and incubated with permeabilized epimastigotes. Hybridization signals were revealed with anti-Digoxigenin and Alexa 488 plus; and nucleus and kinetoplast were labeled with DAPI. Mitotic cells were excluded from the analysis. The ploidy was estimated on a mean of 600 labelled cells for each probe by 3 independent observers. FISH assay showed that the copy number of H49 and JL8 genes varied in the cell population. We found two copies of H49 in 42.2% of the cells, followed by cells with one (28.9%), three (14.8%) and more than three copies (3.8%). While, the proportion of cells with one or two copies of JL8 was very similar (40.8% and 41.9%). Using confocal microscopy, we confirmed the existence of aneuploidy events. The variable copy number of H49 and JL8 genes among individual cells may generate intra-strain heterogeneity and chromosomal mosaicism. Our results could be explained by segmental duplication, but we could not rule out the occurrence of whole-chromosome aneuploidy. We carried out dual-colour FISH on the chromosome TcCh39 using H49 and JL8 probes. **Supported by:**FAPESP, CAPES e CNPq
Keywords: Trypanosoma cruzi, aneuploidy, FISH

PV-024 - **DNA replication in the binucleated protozoan parasite Giardia lamblia**

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Giardia lamblia is a binucleated protozoan containing complete copies of the genome in both nuclei. However, little is known on how this organism handles with DNA replication in each nucleus, which challenges the synchronization of replication during the cell cycle. In this study, we used modern molecular biology techniques to investigate DNA replication in the binucleate trophozoite. Firstly, we pulsed cells with CldU and chased it with IdU, isolated and combed DNA, and visualized the thymidine analogs with green and red antibodies, respectively. Our analysis of replication kinetics shows that the mean replication rate in Giardia was 3.64 ± 1.32 kb.min-1 and higher than in *Trypanosoma cruzi* (2.05 kb.min-1) and *Leishmania donovani* (2.37 kb.min-1). Similar, the mean inter-origin distance was slightly higher in Giardia (IOD = 335.7 ±36.49 kb) compared to other unicellular protozoa parasites. We also estimated the minimum number of origins (MO) required to duplicate an entire chromosome within the S-phase duration. We observed that trophozoites use, on average, 1.55 times more origins than the minimum needed indicating that Giardia activates few backup origins during DNA replication. Further we analyzed the replication patterns in the two nuclei after consecutive dual-pulse labeling with CldU and IdU. As trophozoites attach to surfaces with the ventral disc facing down, this allowed us to establish a basis for nuclear positioning as right or left considering the dorsal view of attached parasites. Replication occurred concerted in 78.5% of cells (N = 388) meaning that both nuclei replicate concomitantly (62.5% showing both nuclei yellow, 7% red, and 9% green), while the remaining cells (21.5%) exhibit variable patterns. Curiously, in this last group 7.5% of cells had at least one nucleus without replication activity. In conclusion, our findings evidence a stunning replication synchronicity between both nuclei in Giardia lamblia, consequence of a smoothie DNA replication **Supported by:**CNPq, and FAPESP **Keywords:**genome duplication, two nuclei, intestinal parasit

PV-025 - ***In vitro* activity of crystal violet structural analogues for drug repositioning against trypanosomatid parasites**

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Trypanosoma cruzi, the causative agent of Chagas disease, uses proline (Pro) as main carbon source when glucose is not available. Pro is also involved in many essential biological processes, such as differentiation and cellular invasion. We have reported that the permease TcAAAP069 mediates Pro uptake and it is involved in *T. cruzi* survival. AAAP transporters family is present in all trypanosomatid parasites and is low represented in mammals. Recently, we have validated TcAAAP069 as one of the targets of crystal violet (CV), a compound used for several years in blood banks to eliminate *T. cruzi*, and then we applied a virtual screening approach in order to identify drugs approved for clinical use that might have similar effects to CV. The antihistamines loratadine and cyproheptadine; and the antibiotic clofazimine inhibited Pro transport and had trypanocidal effect with IC_{50} values between 1 and 13 µM in trypomastigotes and amastigotes.
We also evaluated the antiparasitic effects of the drugs in *Trypanosoma brucei* and *Leishmania donovani*, the causative agents of human African trypanosomiasis and visceral leishmaniasis, respectively. All mentioned CV analogues presented activity against *T. brucei* procyclic trypomastigotes and *L. donovani* promastigotes, with IC_{50} values between 1 and 44 µM. Moreover, preliminary tests on *L. donovani* with cationic liposomal formulations of the drugs showed that drug-loaded liposomes had a higher leishmanicidal activity than free compounds. LdAAP24 and TbAAT6 are the orthologous genes of TcAAAP069 in *Leishmania* and *T. brucei*, therefore the Pro transport inhibition of CV analogues will be evaluated as possible mechanism of action of such drugs.
In conclusion, CV structural analogues are promising anti-trypanosomatid compounds, in addition to being approved drugs used in humans, known as repurposed drugs, which could significantly reduce the requirements for its possible application in the treatment of neglected diseases. **Supported by:**Global Challenges Research Fund (GCRF; MR/P027989/1), Agencia Nacional de Promoción Científica y Tecnológica (FONCyT PICT 2015–0539 and 2018-1801 and 2018-1871), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) **Keywords:**Trypanosomatid parasites.drug repurposing.proline transport

PV-026 - **Identification of a mitochondrion-cell membrane tethering complex in *Toxoplasma gondii***

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Toxoplasma gondii possess a single and dynamic plant-like mitochondrion which is validated as a drug target. This organelle changes its shape during the lytic cycle of the parasite, being predominantly in a peripheral distributed lasso-shape in intracellular parasites and collapsed in extracellular ones. This dynamic is poorly understood since Toxoplasma lacks most of the canonical homologues for the mitochondrial fission and fusion pathways. Our laboratory described a unique outer mitochondrial membrane protein named **LMF1** (***L***asso ***M***aintenance ***F***actor ***1***) that is an interactor of the parasite's homologue for the fission related protein Fis1. Intracellular parasites lacking LMF1 fail to form the lasso-shaped mitochondrion. These mutant parasites also present defects in mitochondrial division and a proliferation phenotype, showing that the correct positioning of the mitochondrion is important for intracellular growth. As LMF1 has no critical domains, we are interested in understanding the proteins that helps LMF1 in the process of tethering the parasite's mitochondrion to the parasite's periphery. Using a yeast two-hybrid screen we were able to narrow down LMF1 putative interactors by generating dual tagged cell lines for confirmatory co-immunoprecipitation assays from parasites. Among these proteins we found IMC10 (TGGT1_230210) as one of the LMF1 interactors. IMC10 is a component of the inner membrane complex, an organelle that lies underneath the plasma membrane and it is a possible link of the mitochondrion with the parasite's pellicle. Using a tetracycline-inducible system we were able to knockdown IMC10 and by immunofluorescence microscopy we observed that, without IMC10, the parasite presents abnormal mitochondrial shape and distribution to the daughter cells during division. Given that, we are showing for the first time that LMF1 mediates the contacts with the mitochondrion and parasite pellicle by protein-protein interaction with IMC10. **Supported by:**NIH/NIAID Project # 5R01AI149766-02
Keywords: Protein-protein interactions.tethering complex.mitochondrion

PV-027 - **Generation of *Trypanosoma brucei* cell lines expressing *Trypanosoma cruzi*'s histidine degradation pathway**

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Trypanosoma cruzi is the etiologic agent of Chagas disease, which is transmitted by triatomine insects. As the life cycle of *T. cruzi* needs an invertebrate vector and a vertebrate host to completion, this parasite is highly adapted to different environments. For example, when glucose is limited in the insect vector's gut, *T. cruzi* can degrade amino acids producing ammonia. Histidine (His) is present in high concentrations in the insect's gut and the parasite can use it to generate ATP through its conversion in glutamate. The coding sequences for the first two enzymes and putative coding sequences for the last two enzymes are present in *T. cruzi*'s genome. However, they are absent in *Trypanosoma brucei*, which makes this parasite a useful model to investigate the biological role of this pathway. We transfected *T. brucei* procyclic form with two plasmids that allow constitutive expression of histidine ammonia-lyase (*Tc*HAL) and urocanate hydratase (*Tc*UH). We confirmed the presence of the enzymes' coding sequences by polymerase chain reaction (PCR), the expression of the enzymes by Western blotting and that the enzymes were expressed in their active forms by enzymatic activity assays. Simultaneous expression of TcHAL and TcUH in procyclic *T. brucei* affects the parasite proliferation rate while their individual expression does not affect parasite proliferation. Also, parasites expressing TcHAL and TcUH simultaneously are not able to use His to maintain cell viability during severe nutritional stress and the presence of urocanate, the product of *Tc*HAL, drastically reduces cell viability when compared to the negative control. Analysis of the complete His degradation pathway in *T. brucei* can contribute to elucidate which evolutionary advantages it brings to *T. cruzi´*s life cycle and provide valuable insights to understanding the parasite metabolism.
Supported by:CAPES **Keywords:**cruzi, brucei, histidine metabolism

PV-028 - **Is the transmembrane glycoprotein p67 a functional lysosomal hydrolase in *Trypanosoma brucei*?**

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p67 is a type I transmembrane glycoprotein of the terminal lysosome of African trypanosomes. p67 is synthetized as initial gp100 glycoprotein that in the lysosome is cleavage to N-terminal (gp32) and C-terminal (gp42) subunits that remain noncovalently associated. Cleavage is blocked by either inhibition or RNAi silencing of the lysosomal protease TbCatL. Other than death, the only phenotype of p67 knockdown is a grossly enlarged lysosome. p67's exact function is unknown, but recent bioinformatic surveys indicate it is the founding member of a phospholipase B-like (PLBL) subgroup of the NTN hydrolase superfamily. PLBLs are found in Eukaryota and p67 models precisely on the crystal structure of the murine orthologue. NTNs autoactivate by internal cleavage at a nucleophilic residue to generate alpha (gp32) /beta (gp42) subunits, which remain non-covalently associated. The N-terminal residue of the beta subunit (Cys, Ser or Thr) then serves as the catalytic nucleophile for hydrolysis reactions. The conserved alpha/ beta cleavage site in p67 is C241/S242. Using a combination of RNAi^{R} p67 constructs in p67 RNAi cell line background it is observed that wildtype (CS) RNAi^{R} p67, but not mutant (AA), is able to rescue cell growth under p67 RNAi induction. The AA mutant is still cleaved in a TbCatL-dependent manner to generate gp32/gp42 subunits, but the gp42 polypeptide is slightly longer at the N-terminus. Thus, p67 activation involves upstream trans-proteolytic cleavage within the gp32/gp42 linker region followed by downstream auto-activation at the CS junction. The precise hydrolytic activity of the PLBL family is under debate. However, preliminary 'omic' data in knockdown cells suggest p67 has amidase activity, either ceramidase or peptidase. Multiple p67 orthologues are found in other parasitic protozoa including *Entameoba*, *Giardia* and *Trichomonas*. Thus it is likely that these hydrolases function as virulence factors in many parasitic diseases. **Supported by:**NIH/NIAID (US) AND UB JSMB (US) **Keywords:**transmembrane glycoprotein p67.phospholipase B-like.NTN hydrolase superfamily

PV-029 - **Delineation and titulation of synthetic peptide as a target for diagnosis of Trypanosoma evansi.**

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Trypanosoma evansi has an essential internal membrane system for removing and adding membrane proteins. In parallel, the recycling system ensures that proteins, including Surface Variant Glycoprotein (VSG) and Surface Invariant Glycoprotein (ISG), return to the surface to maintain the coating. The main evasion strategy of the immune system is antigenic variation, where VSGs undergo frequent remodeling. Unlike these, the ISGs do not show this variation, making them potential targets for diagnosis. The objective was to evaluate the production of polyclonal antibodies against the synthetic peptide of ISG, testing its use as a target for specific diagnostic tests for *T. evansi*. For this, the coupling reaction of KLH to the synthetic peptide was performed. The peptide/KLH conjugate was used in the production of polyclonal anti-peptide antibodies. Two Wistar rats (*Rattus norvegicus*) were used. The immunization protocol involved 5 intraperitoneal injections, FAC and FAI with an interval of 21 days. At the end, whole blood was collected to obtain serum. Antibody detection was performed by indirect immunofluorescence test. Positive control serum (*T. evansi* in formaldehyde 4%) and negative control serum (PBS) were diluted 1:80. The anti-peptide serum was tested at dilutions from 1:40 to 1:1280. The reading of the slides was performed under an epi-fluorescence microscope. The fluorescence emitted by the slide with fixed antigen was identified in titrations from 1:40 to 1:320. Antibody production depends on the animal's immune response. Despite decades of trying to create a comparable protein detection method, no other system (phage display, aptamers, etc.) has achieved the specificity of antibodies created from an animal's immune system. The results suggest that further studies should be carried out to confirm the immunogenicity of the peptide. **Keywords:**epitope, ISG, Surra

PV-030 - **MOLECULAR SCREENING OF LEISHMANIINAE CLINICAL ISOLATES OBTAINED FROM HUMAN CASES OF VISCERAL LEISHMANIASIS TO PERFORM COMPARATIVE GENOMICS**

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Visceral Leishmaniasis (VL) is a vector-borne dise[illegible]. Brazil. It is a neglected disease, largely associated with lack [illegible]oreover, occurrence of species other than *Leishmania* (e.g. m[illegible]*eptomonas*) in cases of leishmaniasis has been reported be[illegible].y that such parasites may be involved with disease develop[illegible]al isolates obtained from patients diagnosed with VL in Ser[illegible]o be analyzed through Whole-Genome Sequencing (WGS) i[illegible]s had been previously screened by means of molecular char[illegible]quencing, morphological analysis and molecular phylogene[illegible]s displayed morphological and phylogenetic differences when[illegible]nstead, they were phylogenetically more closely related to the [illegible]on-infective to humans, suggesting the presence of still unidentified [illegible]mples were further evaluated and screened to meet criteria required for V[illegible]s polyclonal samples from clinical isolates as well as 28 obtained through si[illegible]inical isolate cultures. Forty two samples were genotyped as *Crithidia*-like, while 9 wer[illegible]belonging to genus *Leishmania*. DNA extraction was performed and DNA integrity was asse[illegible]y agarose gel electrophoresis. Lastly, quantification with Qubit fluorometer was employed. Following next-generation sequencing of the samples, results shall be further analyzed through comparative genomics as to characterize this new, potentially emerging *Crithidia-like* parasite. **Supported by:**Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP grant 2016/20258-0, scholarship 2019/24764-6)**Keywords:**visceral leishmaniasis, next-generation sequencing, crithidia-like

PV-031 - **Distribution of active chromatin profile in *Trypanosoma cruzi* epimastigotes and metacyclics forms using FAIRE-seq**

LIMA, A.R.J.[1]; POUBEL, S.B.[1]; ROSÓN, J.N.[1]; COSTA-SILVA, H.M.[1]; SILVA, H.G.D.S.[2]; SILBER, A.M.[3]; SABBAGA, M.C.Q.B.E.[1]; DA CUNHA, J.P.C.[1].
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Trypanosomes' protein-coding genes lacks dedicated promoters and are organized into co-directional gene clusters with unrelated function, resulting in a polycistronic gene transcription unit (PTU). Transcription start and termination sites are believed to be in the intergenic regions between two divergent (dSSR) or convergent (cSSR) PTU, respectively, presenting several epigenetic alterations that regulates transcription. Ribosome profile analysis of *Trypanosoma cruzi* showed more genes being translated in epimastigotes (EPI) than in metacyclics (META). Furthermore, alterations in nucleus and chromatin structures are observed during its life cycle, with EPI showing higher amount of euchromatin. To explore the chromatin changes along *T. cruzi* cycle, FAIRE-seq (Formaldehyde-Assisted Isolation of Regulatory Elements) assays were performed. Reads were preprocessed and mapped to Dm28c strain genome using GATK Best Practices *pipeline*. Alignment ambiguities were removed using Q10 score. Custom scripts and UTRme were used to obtain additional genomic annotation; deepTools 3.3.0 was used for sample normalization and make plots. In general, EPI forms presented higher euchromatin content (expressed as RPGC) than META forms. Combining the FAIRE data with gene expression level (TPM) revealed a positive correlation. At dSSR and cSSR, FAIRE enrichment was observed on both life forms, being higher compared to the whole PTU, and significantly augmented on EPI. Noteworthy, the multigenic families MASP, GP63, mucin and trans-sialidase exhibited distinct FAIRE landscapes in contrast to non-multifamily genes. Additionally, the open chromatin profile marks the difference between core and disruptive genomic compartments. Interestingly, the loci of tRNA genes are greatly enriched by active chromatin mainly at EPI forms. FAIRE data highlights important structural chromatin changes during *T. cruzi* differentiation cycle, reflecting the overall transcriptional state of each analyzed life form. **Supported by:**Fundação de Amparo à Pesquisa do Estado de São Paulo; 2019/19690-3 **Keywords:**Epigenetics, Bioinformatics, tRNA

PV-032- **Effect of ionizing radiation exposure in the cell cycle, morphology and growth of *Trypanosoma cruzi***

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Ionizing radiation induces DNA damage and chromosomal rearrangements in T. cruzi. To investigate the gamma radiation effects on the organization of T. cruzi genome, epimastigotes of CL Brener were exposed to doses of 100-500 Gy of gamma-radiation and then cloned by serial dilution. Eighteen clones were recovered and analyzed for growth in culture and the karyotype by pulsed-field gel electrophoresis. Clone (F9) was isolated from a cell population exposed to a radiation dose of 500 Gy and showed changes in morphology and growth, affecting the clone expansion and culture maintenance. In addition to slow growth, clone F9 showed morphological changes in cell body and flagellum which seems to be thinner and shorter, affecting the motility of parasite. Using Giemsa staining and anti-flagellin antibody, we observed a partial detachment of the flagellum from the cell body and presence of free flagella in the medium. Transmission electron microscopy analysis revealed that in both clone F9 and wild-type (WT) parasites, the flagellum emerges from the flagellar pocket and the axoneme structure remains unaltered. However, the flagellum of clone F9 is detached from cell body after emerging from the flagellar pocket that affected the mobility of parasite. In WT epimastigotes the kinetoplast is located between the nucleus and the flagellum emergence site, while in clone F9 the kinetoplast and flagellar pocket are laterally arranged in relation to the nucleus. The parasite's nucleus-kinetoplast disposition could explain the less wider cell body of F9 cells. To understand the growth deficit, we analyzed the cell cycle of cells arrested in the G1 phase using hydroxyurea-induced cell-cycle synchronization. The G1-phase duration was significantly reduced in F9 cells. Taken together, our results suggest the presence of mutations in the protein genes of flagellum attachment zone (FAZ). This region controls cell morphology by regulating cell length, organelle position and cell division. **Supported by:**FAPESP. N 2017/23312-9 **Keywords:**Ionizing radiation, flagellum, morphology

PV-033 - **Standardization of cutting-edge techniques for the study of T. cruzi replication dynamics**

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LABORATÓRIO DE CICLO CELULAR - INSTITUTO BUTANTAN, SÃO PAULO - SP - BRA

DNA replication is a process that ensures accurate DNA duplication and timely genetic material inheritance. In the case of *Trypanosoma cruzi*, Chagas's disease etiological agent, two very distinct life forms are present in each host, presenting a different replication and infection pattern, indicating that DNA replication dynamics play a significant role in the infection evolution. In order to better understand DNA replication in this parasite, two different techniques are under standardization in the laboratory: chromatin immunoprecipitation followed by Illumina sequencing (ChIP-seq), which allows identifying DNA sites bound by proteins of interest, and DNAscent, that is based on BrdU incorporation into *T. cruzi*'s DNA followed by Nanopore sequencing, allowing the detection on BrdU enriched regions in the genome and then the identification of replication fork direction and potential origins of replication sites, where DNA replication is initiated. Among the components of the pre-replication machinery in this parasite are Orc1Cdc6 and Orc1b, and, aiming to better understand the DNA replication event, these proteins were individually tagged, using a 3xTY1 tag in Cl Brener cell lineages, and submitted to a ChIP-seq experiment. The protein-DNA binding intensity profile of the two proteins was generated for triplicates using two different *T. cruzi* genomes for comparison (Cl Brener and TCC) and is currently under analysis. For the DNAscent technique, synchronized and non-synchronized cultures of Cl Brener were incorporated with BrdU during early-S phase and the extracted DNA was submitted to single cell sequencing, using the Oxford Nanopore Minion sequencer. BrdU enriched regions were detected and are now under evaluation. After all protein binding regions and BrdU enriched regions are processed, it will be possible to validate the establishment of both techniques and better understand the replication dynamics in *T. cruzi*. **Supported by:**Projetos FAPESP 17/07693-2, 20/00694-6, 13/07467-1 **Keywords:**T. cruzi,ChIP-seq,DNAscent

PV-034 - **Histone H2B.V demarcates strategic regions in the Trypanosoma cruzi genome**

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Trypanosoma cruzi is a flagellate *Trypanosomatid* that causes Chagas disease. It transits between its vertebrate and invertebrate hosts, constantly alternating its replicative and infective forms. Among life forms, studies on global transcription showed different transcript levels, which is accompanied by changes in chromatin conformation. Recent studies have elucidated epigenetic components associated with gene regulation present in the chromatin of trypanosomatids that change throughout the life and cell cycle of these organisms. Our group identified, by quantitative proteomics, that several proteins associated with chromatin (including a variant of histone H2B- H2B.V) are differently abundant in *T. cruzi* life forms. These results, however, neither allowed to specifically explore the RNA Polymerase II location in the genome, nor its relationship with histone variants deposition. We performed H2BV and H3 ChIP-seq and observed that 91.3% of H2B.V-enriched peaks were located at dSSR, and only 8.7% were found at non-SSR in the CL Brener assembly; however, not all dSSRs contained H2B.V enrichment. These latter usually flank monocistrons and small polycistrons. Among the H2B.V peaks located at non-SSRs, those were found mainly close to tDNAs and between regions of conserved (mostly protein-coding genes) and disrupted (non-syntenic regions, mostly virulence factors) genome compartments. Currently, we obtained parasites expressing RPB9 (an RNA Pol II subunit) and H4.V tagged, by CRISPR Cas9, to investigate their genomic location by ChIP-seq assays, in order to map the RNA Pol II start and ending regions. These results will be compared to global run-on assays to elucidate the T. cruzi transcription start and terminal regions. **Supported by:**CAPES e FAPESP (17/06104-3 ; 2018/15553-9) **Keywords:**Trypanosoma cruzi, Variant histone, ChIP-seq

PV-035 - **Using CRISPR-dCas9 system as tool for epigenetic studies in *Trypanosoma cruzi***

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In the CRISPR-Cas9 system, the Cas9 is guided by an sgRNA to the target DNA, where it is able to generate a double strand break. The dead Cas9 (dCas9) can bind DNA but is unable to cleave it. So, dCas9 has been used as tool in epigenetic studies. Some aspects of gene expression are unclear in *Trypanosoma cruzi*, which causes Chagas disease. To improve the knowledge about proteins associated with specific chromatin regions in *T. cruzi*, this work aimed to generate a parasite expressing dCas9. To do that, pLEW13-Cas9, a vector carrying Cas9 gene, was used as substrate in mutagenesis reactions generating a vector carrying dCas9 gene (pLEW13-dCas9). The pLEW13-dCas9 was confirmed by Sanger sequencing and used to transfect wild type (WT) epimastigotes of the CL Brener strain. After six weeks of selection, the DNA of WT and transfected ($dCas9^{FLAG}$) parasites was extracted and used to amplify the dCas9 sequence. It showed that only transfected culture amplifies the entire dCas9 gene. To verify if dCas9 was being expressed in the $dCas9^{FLAG}$ parasites, a Western blot was performed. A band for dCas9 was observed only for $dCas9^{FLAG}$ parasites. Since these results suggested that transfected culture was expressing dCas9, these parasites were serial diluted in order to obtain clones. After 20 days, 11 clones were selected and tested by PCR and Western blot. As expected, all clones were carrying dCas9 gene and expressing the protein. Hereafter, it was analyzed the growth profile and cell cycle progression of $dCas9^{FLAG}$ parasites. For most of the clones, the presence of dCas9 gene did not compromise the basic functions of the cell, once WT and $dCas9^{FLAG}$ parasites have the same growth rate and cell cycle progression under normal culture conditions. Ongoing trials aim to use $dCas9^{FLAG}$ parasites in locus-specific chromatin immunoprecipitation which may contribute for a better understanding of factors involved on the establishment and maintenance of gene silencing and activation in *T. cruzi*. **Supported by:**FAPESP 2019/19834-5 e FAPESP 2018/15553-9 **Keywords:**dCas9, Trypanosoma cruzi, epigenetic

PV-036 - **Investigating the transcription and regulatory machineries associated to tRNA genes (tDNAs) in *Trypanosoma cruzi***

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The machinery involved with tDNA transcription in eukaryotes is dependent of the complexes TFIIIC, TFIIIB and RNA polymerase III (RNAP III). In *Saccharomyces cerevisiae*, six proteins form the TFIIIC complex, namely TFC1, TFC4, TFC7, TFC3, TFC6 and TFC8, while the proteins constituents of the TFIIIB complex are BRF1, BDP1 and TBP. The RNAP III contains 17 subunits, namely RPC1, RPC2, RPC40, RPC19, RPB6, RPB5, RPB8, RPB10, RPB12, RPC11, RPC17, RPC25, RPC82, RPC53, RPC37, RPC34, and RPC31. The TFIIIC complex and RPC17/RPC31 subunits of RNAP III have not been identified in *T. cruzi* and little is known about the expression regulation of tDNA in these parasites. Recently, our group observed, by FAIRE-seq technique, that the regions of active chromatin (euchromatin) associated with these genes are more enriched in epimastigote (EPI) than in metacyclic trypomastigote (MT) forms. To better explore these findings and understand how the tDNA transcription occurs in *T. cruzi,* we identified the proteins associated with tDNAs expression as well as evaluated their expression levels at EPI and MT forms. Thus, different in silico approaches (BLAST, DELTA-BLAST and HMMER) were used to identify protein homologs of the tDNA machinery and the translatome dataset on TritrypDB was used to evaluate their expression levels in parasite life cycle. We found all 17 subunits of RNAP III, including RPC17 and RPC31, which are annotated as hypothetical protein. Only two (TFC1 and TFC4) out of the six proteins from the TFIIIC complex were identified suggesting that either this complex works without some members or that it is composed of evolutionary distant proteins not yet identified. All components of TFIIIC, TFIIIB and RNAP III identified are up regulated in EPI than MT. Furthermore, we generated parasites expressing tagged TFC1 by CRISPR-Cas9 to study the proteome around tDNAs using immunoprecipitation assays to better understand the tDNA transcription and their regulatory process in *T. cruzi*. **Supported by:**Fundação de Amparo à Pesquisa do Estado de São Paulo - Processo nº 20/02708-4 **Keywords:**Trypanossoma cruzi, tDNA, transcription

PV-037 - **Evaluation of Trypanosoma cruzi infection in the central nervous system by qPCR and bioluminescent imaging**

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In Chagas disease, cardiac and digestive disorders are the most investigated, however, the damage to the central nervous system and the migration of *T. cruzi* through the brain are less explored. The aim of this study was to evaluate in BALB/c mice, the progression and distribution of *T. cruzi* infection through different regions of the brain. Mice were inoculated with 10^5 trypomastigotes of Dm28c-wild type (WT).The anterior and posterior region of the encephalic cortex, cerebellum and encephalic bulb were collected at the 3, 5, 10 and 15 days after infection (DPI). To quantify *T. cruzi* in the tissues, genomic DNA was purified to perform the quantitative PCR (qPCR), using primers directed to the satellite DNA. Parasites were not detected in most tissues at the 3 DPI; at the 5 DPI, the average of all the brain regions displayed 1.1 equivalent parasites (par. eq.)/µg DNA; at the 10th and 15th DPI, displayed 207.7 and 8910.6 par. eq/ µg DNA, respectively. The most infected regions were: (i) posterior cortex, (ii) frontal cortex, (iii) encephalic bulb and (iv) cerebellum. To perform the *in vivo* bioluminescent imaging, the infection rate of Dm28c WT and Dm28c expressing luciferase (Dm28c-Luc) was evaluated in LLC-MK2 infected cells. The cells were fixed and stained with Giemsa at 24, 48 and 72 hours after infection. The infection rate of the Dm28c-Luc and Dm28c-WT were similar. Thus, mice inoculated with 10^6 trypomastigotes of Dm28c-Luc were evaluated at 1, 7 or 14 DPI by the *in vivo* imaging system. Intense bioluminescent/infection foci was observed in the abdomen at the 7 DPI. Small foci were observed in the head at the 14 DPI. *Ex vivo* evaluation showed infection/bioluminescence in 60% of the brains, at the 14 DPI. The qPCR data suggest that *T. cruzi* was able to disrupt the blood-brain barrier early in the infection, increasing the parasite load in the brain at the 15 DPI, corroborated by the bioluminescent imaging. **Supported by:**PIBIC-Fiocruz-CNPq **Keywords:**Trypanosoma cruzi, Brain, mice

PV-038 - ***Cytochrome b* phylogeny suggests the occurrence of a new species of *Triatoma brasiliensis* complex (Hemiptera, Reduviidae, Triatominae) in State of Rio Grande do Norte, Brazil.**

DA SILVA, A.N.B.[1]; DE SOUZA, R.D.C.M.[2]; ALMEIDA, C.E.[3]; CARVALHO, S.A.P.[4]; HONORATO, N.R.M.[4]; DIOTAIUTI, L.G.[2]; CUNHA, J.L.R.[4]; BARTHOLOMEU, D.C.[4]; BRITO, C.R.D.N.[1]; DA CÂMARA, A.C.J.[1]; GALVAO, L.M.D.C.[1].

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Recently, in the state of Rio Grande do Norte (RN), specimens of triatomines of the genus *Triatoma* were captured in the wild environment cohabitating with *Triatoma brasiliensis* in rocky outcrops. These samples presented morphological characteristics similar to *T. brasiliensis* and *T. petrocchiae*. In this work, we evaluated the phylogenetic relationships of this n. sp. with the members of the *T. brasiliensis* species complex by genetic parameters. We also comparatively analyzed sequences of the mitochondrial *cytochrome b* gene from *T. brasiliensis* and specimens of *Triatoma* n. sp with sequences from members of *T. brasiliensis* species complex, in addition to some taxa of other groups deposited in GenBank. Phylogenetic reconstruction was inferred by the maximum likelihood method, targeting a fragment of cytochrome b gene (432 pb). Node support was estimated using 1,000 bootstrap pseudo-replicates (PR-B). The phylogenetic reconstruction showed branches supported by PR-B values >70. We observed a well-supported clade (PR-B = 89) referring to species of *T. brasiliensis* species complex, including *Triatoma* n. sp. as a member of this monophyletic group (PR-B = 100). All six specimens of *Triatoma* n. sp. were grouped into a single clade as a sister species of *T. petrocchiae* (PR-B = 77). The values of genetic distance between *T. petrocchiae* and *Triatoma* n. sp were estimated by the Kimura method 2-Parameter (K2-P), having been higher (all K2-P > 0.15) than the ones for well-recognized species, such as *R. robustus* and *R barretti* (all < 0.11). Our results demonstrate, therefore, the emergence of an independent evolutionary unit within the *T. brasiliensis* species complex. **Supported by:** MCTI/CNPq/MS-SCTIE-Decit 404056/2012- 1; MCTI/CNPq/Universal 475572/2013-0; CAPES (Scholarships) **Keywords:**Triatoma brasiliensis, Triatoma petrocchiae, Cytochrome b

PV-039 - **Disruption of active trans-sialidase genes impairs the egress from mammalian host cells and generates highly attenuated *Trypanosoma cruzi* parasites**

CALDAS, G.D.A.B.[1]; DOS SANTOS, N.S.A.[1]; DE CASTRO, J.T.[1]; MÜGGE, F.L.B.[1]; DA SILVA, V.G.[1]; OLIVEIRA, A.E.R.[1]; PEREIRA, M.C.D.A.[2]; CUNHA, J.L.R.[1]; COQUEIRO-DOS-SANTOS, A.[1]; GOMES, D.A.[1]; BARTHOLOMEU, D.C.[1]; MORETTI, N.[3]; SCHENKMAN, S.[3]; GAZZINELLI, R.T.[2]; TEIXEIRA, S.M.R.[1].
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Trans-sialidases (TS) are unusual enzymes present on the surface of *Trypanosoma cruzi*, the causative agent of Chagas disease. Encoded by the largest gene family in the *T. cruzi* genome, only few members of the TS family have catalytic activity. Active trans-sialidases (aTS) are responsible for transferring sialic acid from host glycoconjugates to mucins, also present on the parasite surface. The existence of several copies of TS genes has impaired the use of reverse genetics to study this highly polymorphic gene family. Using CRISPR-Cas9, we generated aTS knockout cell lines displaying undetectable levels of TS activity as shown by sialylation assays and labelling with antibodies that recognize sialic acid-containing mucins. In vitro infection assays showed that disruption of aTS genes does not affect the parasite capacity to invade cells or to escape from the parasitophorous vacuole but resulted in impaired differentiation of amastigotes into trypomastigotes and parasite egress from the cell. When inoculated in mice, aTS mutants were unable to establish infection even in the highly susceptible IFN-γ knockout mice. Mice immunized with aTS mutants were fully protected against a challenge infection with the virulent *T. cruzi* Y strain. Altogether, our results confirmed the role of aTS as a *T. cruzi* virulence factor and indicated that aTS play a major role during the late stages of intracellular development and parasite egress. Notably, mutants lacking TS activity are completely avirulent in animal models of infection and may be used as a live attenuated vaccine against Chagas disease. **Supported by:**CNPQ, FAPEMIG, INCTV **Keywords:**Trypanosoma cruzi, Trans-sialidase, CRISPR-Cas9

PV-040 - **Comparison of lipid droplet formation and eicosanoid metabolism in Brazilian *Leishmania* spp.**

ANDRADE, Y.M.F.D.S.[1]; DE CASTRO, M.V.[2]; TAVARES, V.D.S.[2]; SOUZA, R.D.S.O.[2]; LIMA, J.B.[2]; BOZZA, P.T.[3]; SORGI, C.A.[4]; BORGES, V.[1]; SANTOS, T.D.A.[2].
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Introduction: Lipid droplets (LD) are cytoplasmic organelles responsible for the storage of neutral lipids and for the metabolism of eicosanoids in animal eukaryotic cells. Recent data have shown that LDs can be markers of virulence of intracellular pathogens such as ***Leishmania infantum***. In addition, LDs have been described as organelles responsible for the production of prostaglandin F2α in ***L. infantum,*** a molecule capable of modulating the immune response of macrophages during the parasite-host interaction. Knowledge about the lipid mediators produced by parasites is still quite limited and new approaches are needed to identify which mediators are produced and the effects of these mediators on the regulation of cellular processes. Methods: Herein, we used optical microscopy techniques to evaluate the effect of polyunsaturated fatty acids on the formation of LDs and lipid mediators on the production of brazilian ***Leishmania*** species. In addition, we study in silico and in vitro enzymes involved in the eicosanoid metabolism in those parasites. Results: We found that the LDs of ***L. infantum*** and ***L. braziliensis*** are susceptible to change in the presence of polyunsaturated fatty acids, with the clear effect being that induced by arachidonic acid (AA). However, the presence of polyunsaturated fatty acids did not affect the formation of LDs in ***L. amazonensis***. ***In silico*** analysis indicates COX-2 and PGFS from parasites are very similar according clinical form-related. Regarding the production of lipid mediators, we evaluated the presence of 41 different mediators in parasites treated with AA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in cell extracts and in the parasite supernatant by LC-MS. We identified the presence of 14 bioactive lipids, especially when the parasites were stimulated with AA. Conclusions: Altogether, our data opens new perspectives for understanding ***Leishmania*** biology and for the development of drugs with antiparasitic activity. **Supported by:**Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB). BOL0171/2019 **Keywords:**Lipid droplet, Parasites, Lipid metabolism

PV-041 - **Chasing for chromatin-associated proteins and histone PTMs patterns associated to cell cycle stages in *Trypanosoma cruzi***

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Epigenetic marks such as histone post-translational modifications (PTMs) can alter the chromatin compaction and interfere in the gene expression, DNA replication and repair and cell cycle control. For trypanosomatids, these modifications are associated to the transcription start and ending regions as well as associated to cell cycle phases and DNA damage. The insertion or removal of histones PTMs happens through chromatin modifying enzymes, that depends on cell metabolic products, such as S-adenosyl methionine (SAM) and Acetyl-CoA. Therefore, metabolic alterations and, consequently, the availability of these products that act as substrates and/or cofactors of these enzymes, may be related with the chromatin modulation structure and function through changes in histone PTMs patterns. The aim of the present study is to evaluate quantitatively the global pattern of histone PTMs and the expression of nuclear proteins in different cell cycle phases of *T. cruzi*. Epimastigote cultures were cell cycle synchronized with hydroxyurea (HU) to obtain parasites in the G1/S, S and G2/M transition. Histones from parasites at different cell cycle stages were extracted, propionylated and processed for LC-MS/MS analysis using data independent acquisition (DIA). Our findings showed that, for the 0 h time (G1), there was an increase in histone PTMs for the H4 N-terminal peptides $^{16}QK_{me1}KILR^{21}$, $^{16}QK_{me1}K_{me1}ILR^{21}$ and $^{1}AKGKKSGEAKGTQK_{ac}R^{15}$, compared to unsynchronized parasites. Further analyzes will be realized to evaluate global changes in histone PTMs along the cell cycle. In addition, we are currently evaluating proteomic changes on parasite nuclei along cell cycle. We aim to correlate the levels of metabolic enzymes, especially those producing substrates such as SAM and acetyl-CoA, with the levels of histone PTMs throughout the cell cycle. **Supported by:**Fapesp - 2018/14432-3 **Keywords:**Trypanosoma cruzi, Epigenetic, Cell cycle

PV-042 - **Protein partners and post-translational modifications directly associated with two *Leishmania* homologues of eIF2γ, the core subunit of the translation initiation factor eIF2.**

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The regulation of gene expression in trypanosomatids is mostly mediated by post-transcriptional events, with many of those believed to be involved in the initiation stage of mRNA translation. In eukaryotes, this requires the activity of the eIF2 translation initiation complex. eIF2 functions by mediating the recruitment of the small ribosomal subunit bound to Met-tRNAi. eIF2 is formed by the eIF2α, eIF2β, and eIF2γ subunits, with eIF2γ being the core subunit. Herein, the discovery of two eIF2γ homologues in *Leishmania* sp. is under investigation to define their likely functional distinctions. First, we accessed the expression profile by Western blotting with polyclonal serum able to recognize both homologues. eIF2γ-1, the more conserved in sequence with the mammal counterpart, is approximately 30-fold more abundant than eIF2γ-2 in *L. infantum* promastigotes cells. eIF2γ-1 was also shown to be represented by multiple isoforms in exponential growth phase suggesting changes by post-translational modifications. Next, we performed immunoprecipitation assays, followed mass-spectrometry, using the two ectopically expressed homologues, tagged with an HA epitope. The eIF2γ-1 was found to co-precipitate with the other eIF2 subunits as well as with the EIF2B complex, a classical eIF2 partner. As for eIF2γ-2, neither eIF2β nor the subunits of the EIF2B subunits were found to be efficiently co-precipitated. Other known eIF2 partners, such as eIF5 and D123, were found to co-precipitate with both homologues. They also co-precipitated with protein kinases, including the cell-cycle regulated CRK3. Later, Site-directed mutagenesis studies were performed using the HA-tagged protein, aiming to identify possible phosphorylation sites. Our results are consistent with one or both eIF2γ homologues being involved in novel regulatory events which might be linked with the post translations modifications encountered and, indeed with the newly identified interactions with protein kinases. **Supported by:**FACEPE IBPG-1437-2.02/18 **Keywords:**Gene expression regulation, protein synthesis, eukaryotic Initiation Factor

PV-043 - **Analysis of nascent transcripts in Trypanosoma cruzi**

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Trypanosomatids have a genomic structure organized in polycistronic regions. Specific promoter for each gene have not been described thus, post-transcriptional mechanisms are mainly responsible for gene expression regulation. Nascent transcript could elucidate the real impact of transcription regulation on the expression levels of different CDS, between different polycistronic regions, as well as among transcripts from different RNA classes (rRNA, snoRNA, snRNA and mRNA) in *T. cruzi*. To evaluate that, we obtained nascent transcripts using a Global Run-on (GRO) assay by Br-UTP incorporation in epimastigotes. Transcripts were captured by immunoprecipitation and sequenced using a kit that captures small RNAs by adding a polyA tail, in the Illumina NextSeq assuming 1 x 75 bp. Because most small RNAs ware less than 75 bp, there was a dip in quality towards the end of the reads. Reads processing included: trimming for low quality; universal Illumina adapters and polyA tail removal as well as the first four nucleotides from 5' and short reads (<25 nt). Reads were mapped to *T. cruzi* Dm28c indexed genome (release 46) previously masked for the rRNAs, using Bowtie2. We evaluated transcription at specific genomic regions (CDS, polycistron, tRNAs, snoRNAs and snRNAs) by performing analysis on deepTools and compared it in parallel with transcripts publicly available, *T. cruzi* RNA-seq datasets. Although GRO-seq replicates differ between each other regarding their expression profile, it differ from RNA-seq datasets too, mainly at 5' and 3' UTR from CDSs and at polycistronic start sites. GRO-seq data seems to have mRNA processing. To faithfully evaluate that, we are removing transcripts that harbor spliced leader sequence at their 5' UTR. In summary, we obtained a pipeline to process and analyze nascent transcripts, which allow us to conclude that our dataset is reliable. Future analysis will be carried out to compare among CDS and polycistronic regions, in TPM, compared to RNA-seq. **Supported by:**PROEX (Programa de Excelência Acadêmica) CAPES , 88887.569623/2020-00 **Keywords:**Gro-seq, Trypanosoma cruzi, Nascent Transcripts

PV-044 - **Freeze tag: generation of PF16 tagged and knocked out mutants in *Leishmania (Viannia) braziliensis* stably expressing Cas9 nuclease.**

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Since the genome of *Leishmania* was sequenced, in 2005, the use of gene editing techniques has increased, as part of the effort to understand the biology of these complex organisms. Loss-of-function studies using homologous recombination helped to elucidate the role of different proteins in *Leishmania*. This parasite has a very plastic genome in which re-arrangements and aneuploidy are commonly observed, making homologous recombination even more challenging. In 2017, Beneke et al, generated LeishGEdit, a PCR-based tool for knocking out and tagging lines using CRISPR/Cas9. In this system, a plasmid containing the T7 RNA polymerase and Cas9 coding sequences (pT007) is delivered to *Leishmania* for episomal expression or integration in the β-tubulin locus. *L. braziliensis*, the main causative agent of tegumentary leishmaniasis in Brazil, presents a sequence divergence at the β-tubulin locus which impedes the use of the same vector for integration of Cas9 in the genome. To overcome this limitation, the *L. major* β-tubulin sequences, present in the pT007, were replaced by a *L. (Viannia)* β-tubulin conserved sequence. The integration of this cassette in *L. braziliensis* M2903 genome and the expression of Cas9 and T7 in these parasites was successfully obtained. To test Cas9 activity paralyzed flagella protein 16 (PF16) was successfully knocked out in *L. braziliensis* resulting in a phenotype of immobility in these transfectants. The add-back of this gene was generated using an in-locus add-back strategy in which a cassette containing a C-terminal tagged PF16 coding sequence and a puromycin resistance gene was amplified from tagged mutants' genomic DNA and used to return this gene to the original locus. Phenotypic analysis revealed that add-back parasites recovered their swimming capacity. In conclusion, we successfully generated an *L. braziliensis* line stably expressing functional Cas9 and T7 proteins, a new tool for the CRISPR/Cas9-mediated genome editing in this specie. **Supported by:**Capes, CNPq e FAPESP (2016/23405-4, 2020/00087-2 e 2018/14398-0) **Keywords:**Leishmania Braziliensis, CRISPR/Cas9, Flagella

PV-045 - **A conserved Zinc Finger RNA binding protein is implicated in the control of differentiation of both *Trypanosoma cruzi* and *T. brucei***

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Control of gene expression in Trypanosomatids relies mainly on post-transcriptional mechanisms exerted by RNA binding proteins (RBPs) that control stability, translatability, and localization of the mRNA population within the cell. In *Trypanosoma cruzi,* RNA-Seq analysis comparing gene expression in different life cycle stages showed that the gene TcCLB.506739.99 (TcZC3H12), which encodes a RBP containing a zinc finger motif, is upregulated in epimastigotes in relation to trypomastigotes and amastigotes. Functional characterization using gene knockouts identified a role for this RBP in repressing the differentiation from replicative epimastigotes (the insect stage) to infectious metacyclic trypomastigotes. To further characterize the mechanism of gene expression control by TcZC3H12, yeast-2-hybrid and RNA immunoprecipitation experiments were done. Like other zinc finger proteins from *Trypanosoma*, TcZC3H12 interacts with the MKT1-PBP1 complex. Analysis showed that this RBP is bound to, for example, transcripts that can affect differentiation and multiple metabolic pathways. In *T. brucei*, previous studies with monomorphic parasites showed that the knockdown of ZC3H12 ortholog (Tb927.5.1570, TbZC3H12), did not impact parasite growth. To investigate a role for TbZC3H12 in differentiation, polymorphic parasites, that maintain differentiation capacity, were now used. CRISPR/Cas9 knockout in procyclics (PCs, the insect stage) reduced the parasite growth rate and increased social motility on semi-solid medium. Direct knockout in blood stream forms (BSF) was not possible, but RBP10 overexpression was able to promote differentiation in specific conditions, resulting in BSF that also showed a slower growth rate. To identify the targets of this RBP in *T. brucei*, RNA-Seq of knockout PCs and BSF, and RNA immunoprecipitation analysis of tagged TbZC3H12 are underway. **Supported by:**DAAD (German Academic Exchange Service), DFG (German Research Foundation) **Keywords:**Trypanosoma, RNA binding proteins, parasite differentiation

PV-046 - **Analysis of duplicated Ribosomal Proteins RPS16 and RPL13 in *Leishmania***

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In *Leishmania* parasites most genes encoding ribosomal proteins (RPs) are present as two or more copies in the genome. Some of the duplicated genes code for identical proteins, some for distinct, but all of them carry a divergent untranslated regions (UTRs). These differences at the UTRs or protein coding sequences (CDS) might confer loss or gain of function to the proteins. In this context, our aim was to study RPs S16 and L13 expressed from duplicated genes in *Leishmania major*. RPs S16 transcripts have divergent UTRs and identical CDSs, and L13 copies have, in addition to divergent UTRs, non-identical CDSs. We generated knockout transfectants (KO) of each one of the four genes and transfectants with tagged versions (*myc tag*) of each CDS using the CRISPR/Cas9 system for genome editing. Distinct levels of expression of each copy of S16 (RPS16_90 and RPS16_80) and L13 (RPL13_15 and RPL13_34) were observed; RPS16_90 and RPL13_34 protein copies were always lower than the counterpart. Moreover, the levels of expression of one of the S16 paralogs would go up when the second copy was knocked out, suggesting a paralog compensation to maintain the levels of the protein. Curiously, we failed to obtain RPL13_15 KO when RPL13_34 were *myc* tagged, in both N and C terminus, suggesting that the tag affects RPL13_34 function. Furthermore, KO transfectants were subjected to nutrient deprivation conditions and a recovery period in fresh medium. Cell viability analysis revealed that KO parasites for all the four genes showed increased cell viability after the nutritional stress and recovery period. Immunofluorescence analysis, using the four *myc* tagged transfectants, revealed a similar distribution of the protein throughout the cytoplasm. We are currently analyzing the subcellular distribution of these proteins after stress. **Supported by:**FAPESP (2019/05257-6 and 2018/14398-0) **Keywords:**Leishmania, Ribosomal Protein, Extra-ribosomal function

PV-047 - **Distribution of *Trypanosoma cruzi* and its vectors in a semi-arid area of northeastern Brazil**

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Trypanosoma cruzi is genetically divided into six discrete typing units (DTUs), TcI – TcVI. In the state of Rio Grande do Norte (RN), Brazil, TcI, TcII and TcIII have been found in several vertebrate reservoir and triatomine species. However, this occurrence and distribution within vectors may be underestimated in this area. Therefore, we investigated *T. cruzi* DTUs associated with triatomines and identified bloodmeal sources, in anthropic and sylvatic environments in the state of RN. The genotyping of *T. cruzi* isolates was performed using the mitochondrial cytochrome c oxidase subunit 2 gene, the D7 region of the 24Sα rDNA and the spliced leader intergenic region. The bloodmeal sources of triatomines were identified amplifying the 12S rRNA locus. Five triatomine species were captured, and the most frequent was *Triatoma brasiliensis* (84.3%; 916/1086), which was found in 16 of the 23 municipalities surveyed, and infested all types of environment investigated. The TcI was found in all mesoregions surveyed in 51.5% (17/33) of the culture-positive samples. In contrast, TcII (9.1%; 3/33) was detected in the Central mesoregion, while TcIII (27.3%; 9/33) was found in all mesoregions. The geographic distribution using ecological niche distribution modelling revealed an overlap of different DTUs. *Triatoma brasiliensis* was found infected in all mesoregions and with all three *T. cruzi* DTUs, including mixed infections. The DNA of rodents was found in triatomines infected with either TcI or TcIII, while that of domestic animals and humans was associated with both single and mixed infections. Our findings demonstrate that different DTUs of *T. cruzi* are widely dispersed among triatomines in our study area. The association of *T. brasiliensis* with several different mammalian hosts, as well as overlapping areas with different DTUs, suggests that this triatomine species may have an important role as a vector in both anthropic and sylvatic environments. **Supported by:**CNPq, CAPES **Keywords:**Triatoma brasiliensis, Peridomicile, Blood-feeding

TB-001 - **Miltefosine and Amphotericin B susceptibility in reference strains and clinical isolates of Leishmania spp. responsible for tegumentary leishmaniasis**

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Tegumentary leishmaniasis (TL) is a complex of diseases caused by several species of the protozoan of the genus *Leishmania*. In Brazil, TL is caused mainly by *Leishmania (Viannia) braziliensis, L. (Leishmania) amazonensis* and *L. (V.) guyanensis*, being the first, the most prevalent species. In recent years, leishmaniasis has presented an increasing number of cases, mainly in urban regions. The treatment of TL in Brazil consists in the use of pentavalent antimonials and amphotericin B (AmB), drugs that are considered costly, toxic, and require parenteral administration. Recently, miltefosine (MF) was approved for the clinical treatment of TL in Brazil, although it is not yet available in the National Health System. In this study, we aim to evaluate the *in vitro* susceptibility to AmB and MF in all *Leishmania* species responsible for TL in Brazil and in a panel of 16 clinical isolates of *Leishmania* spp. from a reference center for treatment of TL. The *in vitro* susceptibility to AmB and MF of clinical isolates and species of *Leishmania* were determined in promastigote and intracellular amastigote forms. The results obtained indicated a moderate variation in the susceptibility to these drugs in clinical isolates and in *Leishmania* species in both forms of the parasite. In addition, an AmB resistant line of *L. (L.) amazonensis* is currently selected *in vitro*. These findings will contribute to evaluate the limitations of the use of AmB and the potential of MF as an alternative drug for the treatment of LT in Brazil. **Supported by:**FAPESP (Processo: 2020/01948-1) **Keywords:**Tegumentary leishmaniasis, amphotericin B, miltefosine

TB-002 - **Studies on paromomycin susceptibility and resistance in *Leishmania amazonensis***

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Cutaneous leishmaniasis is a disease that has presented, in recent years, an increasing number of cases in Brazil. The disease is caused mainly by *Leishmania amazonensis* and *L. braziliensis*. The treatment of the disease in Brazil is limited to the use of parenterally administered drugs that induce several side effects. Paromomycin (PM) was already approved as alternative to the treatment of visceral leishmaniasis in Southeast Asia. Although not used in the treatment of cutaneous leishmaniasis, it is important to investigate the potential of this drug against species endemic in Brazil. Here, we selected PM resistant lines of *L. amazonensis* in promastigote and intracellular amastigote forms by stepwise selection and mutagenesis followed by drug selection. To understand the molecular basis of PM susceptibility and resistance, our main goal is to identify and validate genes associated with susceptibility and resistance to PM through whole genome sequencing of these PM resistant lines and clinical isolates of *L. amazonensis* with differential *in vitro* susceptibility, previously characterized by our group (Coser et al., 2021). We are also investigating the accumulation of PM in these resistant lines and clinical isolates, using a PM fluorescent analog. Previous results indicate a direct correlation between PM susceptibility and the accumulation of the drug. This study will contribute to a better understanding of the mechanism of action and resistance of PM that are not completely understood in *Leishmania*, as well as its potential use in chemotherapy of the cutaneous leishmaniasis. **Supported by:**FAPESP (Processo 2019/22175-3) **Keywords:**Leishmania amazonensis.Paromomycin.Resistance

TB-003 - **Could the inositol pyrophosphates (PP-IPs) be involved in DNA repair pathways in the human pathogen Trypanosoma cruzi? Preliminary analyses**

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In model eukaryotes, inositol pyrophosphates (PP-IPs) are involved in a wide range of processes, such as regulation of telomere length and homologous recombination (HR). However, the action mechanism of PP-IPs in pathways related to DNA metabolism is not fully understood. The PP-IPs (IP_7 and IP_8), are synthesized by pathways involving the participation of IP6K and PP-IP5K kinases, respectively. Trypanosomatids, which encompass parasites of great medical relevance, such as *Trypanosoma cruzi* , apparently do not present homologues for PP-IP5K, which make these organisms excellent models for the study of PP-IPs. The goal of this study is to deplete the IP6K gene in *T. cruzi* , generating KO lineages ($IP6K^{-/-}$), and investigate the possible participation of IP_7 in DNA repair pathways. After the 1 st round of genome editing using CRISPR-Cas9 and clone selection, PCR analyzes revealed a single null population ($IP6K^{-/+}$). The second round of transfection is being done to achieve a possible double null population ($IP6K^{-/-}$). After clone selection and isolation, lineages will then be characterized phenotypically, and the absence of IP6K, as well as IP_7 , will be checked by qPCR and PAGE, respectively. Episomal IP6K add-back lineages will be generated to demonstrate the specificity of the assay and eliminate bias due to off targets. $IP6K^{-/-}$lineages and controls (WT and add-back) will be challenged with different genotoxic stress (IR, H_2O_2 , and UV) to induce different types of DNA damage. Growth curves and FACS analyzes will be done to check the proliferation pattern and possible cell cycle arrests. Next, we will establish and compare the recruitment kinetics for HR, BER, and NER repair pathways by IFA and western blot assays using specific antibodies. The possible participation of PP-IPs in DNA repair pathways could provide new routes for developing antiparasitic therapies for *T. cruzi* and other related infectious trypanosomatids. **Supported by:**SAGe-FAPESP, 2020/16480-5 **Keywords:**inositol pyrophosphates, pyrophosphorylation, Trypanosoma cruzi, DNA Damage, DNA Repair, CRISPR/Cas9

TB-004 - **Detecting compound target engagement in living *Leishmania* cells**

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Modern, target-based drug discovery strategies hold the promise of delivering better, safer medicines for leishmaniasis. However, progress in this area has been hampered by the lack of tools for the validation of new chemical entities effective against genetically-validated molecular targets. Here we used bioluminescence resonance energy transfer (BRET) to detect compound target engagement and determine its potency and binding kinetics within promastigotes and macrophage-residing amastigotes of *Leishmania*. Our assay is based on BRET between a donor, the target protein fused to NanoLuc (a small engineered luciferase), and an acceptor, a cell-permeable fluorescent probe that binds to the BRET donor. Displacement of the BRET probe from the target by a competing ligand disrupts energy transfer and directly proves target engagement in living cells. Here, we used the CLK1 protein kinase of *Leishmania mexicana* as a target for validation of this technique. Commercially-available BRET probes did not bind to purified NanoLuc-fused CLK1 *in vitro* or in cell-based assays using genetically-engineered *Leishmania* expressing the fusion protein. Using the purified protein, we identified novel CLK1 ligands from a human kinase ligand library. Based on available structural information, we modified one of these ligands to function as a *Leishmania*-permeable BRET probe. We used this probe in cell-based assays to identify novel compounds that can engage CLK1 in both promastigotes and intracellular amastigotes. We also confirmed that compounds that could bind our target in cells were also able to inhibit its phosphotransferase activity *in vitro*. Finally, we showed that these compounds could kill *Leishmania* promastigotes and intracellular amastigotes, and that overexpression of CLK1 led to resistance, confirming our findings. We expect this assay can be expanded to other genetically-essential targets to expedite the discovery of new anti-leishmanial compounds. **Keywords:**Leishmania.Drug discovery.Protein kinases

TB-005 - **A STRUCTURE-BASED DRUG DISCOVERY PROGRAM TARGETING *LEISHMANIA* GLYCOGEN SYNTHASE KINASE 3**

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Leishmaniasis is a group of neglected tropical diseases caused by parasites from the genus *Leishmania*, which claims over 20,000 lives and affects more than 1 million people every year. Current treatments are not satisfactory – most have severe side effects and require hospitalization. Unfortunately, the development of new drugs to treat leishmaniasis has been hampered by the difficulty to identify valid therapeutic targets. Protein kinases represent promising candidates for drug development against parasitic diseases. Glycogen synthase kinase 3 (GSK3) is a multifunctional Ser/Thr kinase found in all eukaryotes. In humans, there are two isoforms, alpha (GSK3A) and beta (GSK3B), which show both distinct and redundant functions. Counterparts of human GSK3 have been described in *Leishmania*, GSK3a and GSK3b (long and short isoforms, respectively). Both kinases have been identified as genetically-essential for parasite viability, and GSK3b has also been pharmacologically validated. Here we started a target-based drug discovery program to develop potent and selective inhibitors of *Leishmania infantum* GSK3a and GSK3b. In order to identify initial hits, recombinant protein kinases were produced and screened against a set of ~1,400 compounds. Biochemical assays were employed to validate positive hits and to determine IC_{50} and Ki values. Promising compounds were further investigated by phenotypic assays to evaluate its antileishmanial activity and host cell toxicity. We are currently pursuing structural determination of protein-inhibitor complexes to elucidate ligand-binding modes and to guide lead optimization. We expect the data obtained in this work to support a structure-based drug design program to investigate the use of GSK3a and GSK3b as therapeutic targets against leishmaniasis. **Supported by:**Eurofarma, DNDi, FAPESP, Embrapii, CNPq **Keywords:**target-based drug discovery.therapeutic targets.leishmaniasis

TB-006 - **Building tamoxifen/clemastine chimera as a strategy to explore new treatments for leishmaniasis**

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The range of drugs available to treat leishmaniasis are far from ideal: they induce lethal side effects, require special infrastructure due to parenteral administration and some of them have been showing a decrease on responsiveness to treatment. Collectively, these shortcomings make the discovery of new alternative treatments an urgent matter.
Replacing an existing approved medication is an attractive strategy to accelerate drug discovery and, in this context, we have identified both tamoxifen, a known anti-breast cancer drug, and clemastine fumarate**,** an antihistamine drug, as displaying potent anti-leishmanial activity. Both molecules have been proposed to target the same enzyme, inositol phosphorylceramide synthase (IPCS), but also exhibit other pharmacologies. To explore this in greater detail and develop more effective selective compounds, we have built a library of tamoxifen/clemastine hybrids based on the common features shared by these molecules. Following initial screening against *Leishmania major* promastigotes, those with an EC_{50} < 5 μM were selected for further testing against other species of *Leishmania* and preliminary cytotoxicity evaluation against HepG2 cells. The most potent molecule to date has an EC_{50} = 0.27 ± 0.026 μM against *L. major* with a SI > 300. Current efforts are focused on turning this compound into a probe to identify the protein target. This presentation will describe the details of these studies together with the application of the probe in target identification and validation.
Supported by:GCRF-CDT, UKRI **Keywords:**Leishmania.Tamoxifen.Clemastine

TB-007 - **A SYBR GREEN-BASED REAL-TIME PCR ASSAY FOR DISCRIMINATION OF LEISHMANIA INFANTUM AND CRITHIDIA-LIKE PARASITES IN SAMPLES OF HUMAN VISCERAL LEISHMANIASIS**

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Visceral leishmaniasis (VL) affects the bone marrow, spleen, liver and can be fatal if left untreated. In Brazil, VL is caused by *Leishmania infantum* transmitted by sandfly vectors. LV patients in Middle East and India can be co-infected with monoxenous trypanosomatids. Previously, we identify a non-*Leishmania* parasite in an atypical and fatal case of VL in Sergipe, BR. Phylogenomics revealed similarity to *C. fasciculata*, (named as *Crithidia-like*). Correct parasite identification is important for diagnosis and treatment. Thus, here our aim was to standardize a SYBR Green qPCR assay targeting species-specific genes in genomes of *L. infantum* (LinJ31_X) and *Crithidia-like* (LVH60-12060) able to discriminate and determine the parasite load theses parasites in clinical isolates samples obtained from bone marrow aspirates during hospitalizations. Clinical isolates were kept in culture for genomic DNA extraction. Genomic materials from vertebrate hosts (human, dog and cat), from other *Leishmania* species and *C. fasciculata* were also used. As a reference control in the *L. infantum* detection, primers used elsewhere for conserved region in *Leishmania* repeats (REPL) (LinJ31_L42486.1) of VL-causing species were used. The standard curve to quantify the parasite load was calculated based on the genomic DNA mass considering the genome sizes of *L infantum* and/or *Crithidia-like* plus kDNA content equivalent to one parasite. *L. infantum* sample (PP75) amplified only in LinJ31_L42486.1 and Linj31_X. Likewise, *Crithidia-like* sample was only amplified with LVH60-12060F primer. The mass one *L. infantum* and Crithidia-like were 79.2fg and 85.2fg, respectively. The standard curve was built ranging from 10^1 to 10^6 parasites/µL presenting a quantification cycle (Cq) from 30 to 25, respectively in both set of primers. The validation of these primers is still ongoing. New targets for molecular diagnosis can assist in the elucidation of atypical and severe cases of VL. **Supported by:** FAPESP grant 2016/20258-0; CNPq scholarship 133661/2020-2 and CAPES Finance Code 001 **Keywords:**A SYBR green-based real-time PCR, Leishmania infantum, Crithidia-like

TB-008 - **Biology of RNA genes in trypanosomatids**

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Trypanosomatids are unicellular eukaryotes that differ from the rest of the eukaryotes in several aspects regarding RNA metabolism, gene expression regulation and gene organization. Recently, with the advent of long-read technologies (PacBio and Oxford Nanopore Technologies), several trypanosomatid genome assemblies were published using third-generation sequencing technologies which improves genome sequencing contiguity. Even though several efforts were made in order to correctly annotate coding genes and repetitive sequences, non-coding RNA annotation has not been thoroughly assessed. These RNA genes encode functional RNA products and they are often a neglected class of genes in large scale genome analysis probably due to their sequence and structure diversity that require more dedicated annotation. Since these genes do not present the features that define coding genes (e.g. long open reading frames) and instead present limited sequence conservation, classical strategies for gene annotation can not be used. The peculiar mechanisms of RNA metabolism in trypanosomatids and the improved genomes' assemblies prompted us to study how the non-coding RNA genes are organized in these genomes. We used several optimized algorithms depending on the RNA to re-annotate them providing a complete annotation, including the identification of previously undescribed non-coding RNAs as well as the correct annotation of genes that were previously incorrectly assigned. In sum, this work reports a highly curated genome annotation, and unveils the organization of non-coding RNAs in trypanosomatid genome assemblies.

Supported by: Research Council United Kingdom Grand Challenges Research Funder 'A Global Network for Neglected Tropical Diseases' grant number MR/P027989/1. **Keywords:**non-coding RNA genome annotation.genome organization.trypanosomatids

TB-009 - **High Content Screening to Evaluate the Antileishmanial Activity of Drugs against Intracellular Amastigotes**

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Leishmaniasis is caused by several species of the protozoan parasites of the genus *Leishmania*. This disease treatment remains difficult, since the available drugs have shown to be highly toxic, besides several effects has been shown. Thus, new therapeutics are urgently needed. High content screening represents an important tool in the drug discovery process, since it optimizes the chances of finding an active compound from a large number of candidates. So, the aim of this work was to evaluate the antileishmanial activity of some commercial drugs by high content screening targeting intracellular amastigotes. Bone marrow-derived macrophages (BMDMs) were plated in 96-well plates, and then infected with *L. amazonensis* expressing RFP. 3h post-infection, cells were treated with the drugs at 10 µM. Then, image acquisition was made 24h, 48h and 72h post-treatment. It was evaluated the antileishmanial activity of 2560 drugs in the initial screening. After that, were selected 80 compounds showing at least 50% of intracellular amastigotes inhibition when compared to controls (treatment with DMSO 1%). These 80 compounds were re-tested in a second screening, in which 53 compounds maintained the activity. Next, the 53 compounds were diluted in medium and tested again, but only 38 still demonstrated antileishmanial activity. Following, the 38 compounds have determined their EC_{50} and CC_{50} values, at this point 26 compounds that demonstrated EC_{50} < 10 and SI > 10 were selected. These 26 selected compounds were repurchased lyophilized and re-tested against intracellular amastigotes, in this step 18 compounds maintained the antileishmanial activity. After other selection steps based on drug approval by regulatory agencies and route of administration, we selected 9 compounds FDA-approved and orally administered to follow in our *in vivo* studies. **Supported by:**FAPESP - 2017/19040-3
Keywords: Leishmaniasis, High Content Screening, Leishmania amazonenses

TB-010 - **COMPARATIVE TRANSCRIPTOMIC ANALYSIS OF BENZNIDAZOLE RESISTANT AND SUSCEPTIBLE Trypanosoma cruzi POPULATIONS**

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INTRODUCTION: Chagas disease (CD) is an important public health problem in Latin America caused by the protozoan Trypanosoma cruzi. Nifurtimox and Benznidazole (BZ) used for CD treatment, present low cure efficacy, mainly in the chronic phase of CD and several side effects. Then, there is an urgency in the discovery of new drugs for CD treatment and a better understanding of the BZ-resistance mechanisms in T. cruzi. **OBJECTIVES:** We use the RNA seq approach with the Illumina NovaSeq technology to identify and compare the differentially expressed transcripts between BZ-resistant and -susceptible T. cruzi populations from the Tehuantepec strain. **METHODS:** All the cDNA libraries were constructed from epimastigote forms of each population, sequenced and analyzed using STAR for mapping the reads against the reference genome (T. cruzi Dm28c), and EdgeR for differential expression statistical analyses. RESULTS: The analytical pipeline considering an adjusted p-value lower than 0.05 and a fold change greater than 2.0 identified 544 transcripts differentially expressed (DE) between susceptible and BZ-resistant T. cruzi populations. Out of 544 DE transcripts, 460 presented functional annotation, and 84 were assigned as hypothetical proteins. A total of 374 transcripts were upregulated and 170 were downregulated in the BZ-resistant T. cruzi populations. Using this DE dataset, the proteins were further grouped in functional classes according to the Gene Ontology database. We observed that the main transcripts identified are associated with biological processes of pathogenesis, amino acids metabolism, response to toxic substance, proteolysis machinery, cell replication, metabolic process of nitrogen and nucleic acids, cytoplasmic translation, regulation of metabolic process, among others. **CONCLUSION:** In this study, we generated a list of genes differentially expressed in T. cruzi and confirmed that the BZ-resistance phenotype of this parasite is multifactorial and complex. **Supported by:**CAPES, CNPq, FAPEMIG e INOVA FIOCRUZ **Keywords:**Trypanosoma cruzi, RNAseq, drug-resistance

TB-011 - **Assembly, annotation and gene editing of the genome of the PH8 strain of Leishmania amazonensis with focus on multigene families encoding virulence factors**

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Leishmania amazonensis is one of the etiological agents of cutaneous leishmaniasis. Parasite virulence factors have already been studied, as they play a crucial role in the establishment of infection and disease development in the mammalian host. We report the sequencing and assembly of the PH8 strain of *L. amazonenis* combining long PacBio reads, short Illumina reads and synteny data with the *Leishmania mexicana* genome. The final assembly, composed of 34 chromosomes and 44 scaffolds not incorporated, represents a genome of ~ 32 Mb. The annotation of the *L. amazonensis* genome was transferred from the annotation of 8225 genes present in the genome of *L. mexicana*. Multigene families, such as amastins, metalloproteins GP63, A2 protein, cysteine proteases, fatty acid synthases, phosphatase and kinases, were identified using an automated pipeline, in which proteins from others Leishmania species and trypanosomatids interrogated. In addition, amastins and GP63 were characterized. In total, 25 genes encoding amastins were predicted in *L. amazonensis*. The alignment of this genes, using M-Coffee and phylogeny analyzes, using the maximum-likelihood estimation resulted in groupings corresponding to the four sub-classes of amastins (α, β, γ and δ). Analysis of the 11 sequences encoding GP63 showed the conservation of important domains, such as HExxH and SRYD, which are important for protein structure and binding to macrophage surface receptors, respectively. Finally, we tested a CRISPR-Cas9 protocol to generate knockout cell lines of the Miltefosine Transporter (TM) gene of *L. amazonensis* as a proof of concept. Expression of *Streptococcus pyogenes* Cas9 in promastigotes was achieved after transfection with pLDCN, an episomal vector. Alternatively, promastigotes were transfected with recombinant *Staphylococcus aureus* Cas9 ribonucleoprotein complex and an sgRNA. Both strategies were able to disrupt the TM gene in transfected parasites, originating knockout parasite cell lines. **Supported by:**CAPES
Keywords: Leishmania amazonensis.annotation.multigene families

TB-012 - **Novel Benznidazole Derivatives and Functionalized Graphene Oxide Matrix and its interaction with Trypanosoma cruzi parasites**

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Since the discovery of Benznidazole and Nifurtimox in the 60s and 70s and their applications in the combat against Chagas Disease, there have been few advances in the field of developing new molecules that can be used to fight the parasite, both in the acute and chronic phase of the disease. Despite a series of molecules that are currently used in addition to the reference drugs Benznidazole and Nifurtimox, we can cite Posoconazole, Ravuconazole, Itraconazole, Fexinidazole and the Benzofurans Amiodarone/Dronedarone. However, none of these new molecule have been developed or even allowed their singular use as alternative to chemotherapy.
Here, we synthesized seven new Benzinidazole derivatives keeping the nitroimidazole part of molecule, with the intention to evaluate the antichagasic activity of these prodrugs. The nitroimidazolic derivatives were submitted to *in vitro* test against epimastigote form of *Trypanosoma cruzi* and two compounds showed activity very close to Benznidazole at concentration of 5 µM.
A matrix based on Graphene Oxide was also developed and now we are envisioning their use in combination with the synthesized prototypes, in order to verify the activity and cytotoxicity of this therapeutic combination in different T. cruzi life forms. **Supported by:**FAPESP - Processo 00770-4
Keywords: Benznidazole derivatives, Antichagasic activity, Graphene oxide Matrix

TB-013 - **Use of computational approaches in a rational strategy for discovery of a new chemistry entity and repurposing FDA-approved drugs for leishmaniasis treatment by structure-based virtual screening**

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The treatment for leishmaniasis still presents a serious public health problem and its necessary to search compounds that are less toxic, more effective, and able to oral administration. Thus, the aim of this work was to select natural compounds (NAT) for a new chemistry entity (NEC), and trial FDA-approved drugs (F-drugs) to repositioning using the trypanothione reductase from *Leishmania infantum* (TrLi) in a structure-based virtual screening (SBVS) approach. Libraries with 65 NAT and 2104 F-drugs were obtained from ZINC database and computational approaches were used to select the most promising compounds and study their molecular mechanisms of action (MMA). After ADMET analyzes, 6 NAT were selected to SBVS using TRLi as target (PDB: 2JK6 and 4ADW). All of them showed conformations in the active site of enzyme. Next, the most promising compounds (NC2 and NC4) were select to study their MMA in the enzyme-inhibitor complex. Both demonstrated important interactions in the active site, and an estimated Ki of 3.4µM and 1.7µM for NC2 and NC4, respectively, in 2JK6. Corroborating, in 4ADW the Ki estimated was 4.3µM for NC2 and a Ki of 805nM for NC4. In F- drugs, a total of 100 compounds with less binding energy from 2JK6 and 4ADW were selected by SBVS. Thus, in each list, was analyzed the ADMET proprieties, compounds licensed by ANVISA and available on SUS. Finally, 3 compounds were selected for MMA study. Selected compounds demonstrated an estimated Ki of 97.5nM and 461.6nM, 9.0nM and 30.1nM, and 20.9nM and 43.7nM for SUS1, SUS2 and SUS3 in 2JK6 and 4ADW, respectively, and made important interactions with residues of catalytic triad of TRLi. Together, our data demonstrated that SBVS could be an excellent alternative to select promising compounds able to be a competitive inhibitor of TRLi, encourage us continue to investigate theirs effects in *L. infantum* and suggest that these compounds are a potential candidate for leishmaniasis treatment by oral route. **Supported by:**CAPES; CNPq; FAPERJ; IOC/FIOCRUZ **Keywords:**Drug Discovery, Structure-based virtual screening, Trypanothione reductase

TB-014 - **Bone marrow response of dogs naturally infected with *Leishmania infantum* presenting different clinical outcomes**

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Canine visceral leishmaniasis (CVL) can be presented as a severe debilitating or subclinical form of the disease that progression depends on several factors, including the host immunological status triggered by the infection. Our main objective is to characterize the response of bone marrow (BM) cells in dogs naturally infected with *L. infantum* with different clinical outcomes. Initially, the longitudinal analysis of the hematological profile of resistant and susceptible dogs was monitored in a cohort study performed using random-effects models for longitudinal data. Subsequently, 8 resistant and 8 susceptible dogs were selected from the cohort study for reassessment and characterization of the BM cellular immune profile, using a myelogram, and identifying the expression of genes in BM cells using RNASeq. Also, we included in the analysis 8 control uninfected dogs. In the longitudinal analysis of the hematological profile and the blood count of the reassessed dogs, red blood cell counts, hemoglobin, and hematocrit values showed a significant decrease in susceptible dogs. Myelogram evaluation revealed that only susceptible animals showed erythroid cell hypoplasia. In the transcriptome analysis, 425 differentially expressed genes (DEGs) were identified in the samples of resistant dogs and 327 DEGs in the samples of susceptible dogs. The enrichment analyzes revealed that pathways related to DNA repair were negatively regulated in the BM cells of susceptible dogs, while pathways related to cell migration were modulated in susceptible and in resistant dogs. An algorithm based on machine learning identified a set of 4 genes (*EGR2*, *FOS*, *TINAGL1* and *ADCY9*), which exhibited the highest classification power to describe resistant and susceptible dogs. In conclusion, identified alterations in peripheral blood and BM in dogs that develop CVL shown to be associated with the susceptibility profile. DEGs will be validated by qPCR and using functional studies.
Supported by:FAPESB (Universal - Nº 05/2015), CAPES (001) e CNPq (Bolsa de doutorado - 154049/2016-6) **Keywords:**Canine leishmaniasis.spinal response.RNA-Seq

TB-015 - **Drug Discovery and Development against leishmaniasis and Chagas' disease**

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Leishmaniasis and Chagas' disease are infectious diseases caused by parasites from the genus *Leishmania* sp. and species *Trypanosoma cruzi,* respectively. The diseases are manifested in very different ways but do share common facts, including vectorial transmission and affecting mainly underserved communities in developing countries, mostly in tropical areas of the globe. Hence, they are included in the group of diseases collectively called Neglected Tropical Diseases. Unfortunately, the drugs available to treat these diseases are not effective, toxic, and/or expensive, and there is a consensus that new and better therapies are a clear unmet medical need. At UC San Diego, we have developed a drug discovery pipeline that enables the screen of libraries of compounds to identify molecules with antiparasitic activity in cell-based assays targeting the relevant forms associated with human disease. We have currently molecules in different stages of development. Our goal is to develop pre-clinical candidates. **Keywords:** Drug Discovery, Drug Development, leishmaniasis, chagas disease

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