PV017 - **FUNCTIONAL CHARACTERIZATION OF A HISTIDINE TRIAD PROTEIN-LIKE AND CALMODULIN IN *PLASMODIUM FALCIPARUM***

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Calcium signaling and calcium-activated proteolysis have fundamental roles in many aspects of the *Plasmodium falciparum* life cycle. Calmodulin and Histidine-triad (HIT) proteins are involved in calcium homeostasis in mammalian cells. Here, we overexpressed the *Plasmodium falciparum* calmodulin (PfCaM) and a HIT-like protein (PfHint-1) seeking to clarify their functions in the malaria parasite. PfHint-1 is expressed in erythrocytic stages and partially colocalizes to the endoplasmic reticulum (ER). Parasites overexpressing PfHint-1 displayed lower [Ca^{2+}] in the ER store and the ion homeostasis was altered, as higher Ca^{2+} influx from the extracellular medium was observed after the depletion of Ca^{2+} in the ER. PfCaM overexpressing parasites displayed a higher cytosolic calcium rise after the challenge with a calmodulin inhibitor, suggesting that PfCaM is an important Ca^{2+} homeostasis modulator, workingas an intracellular buffer in *Plasmodium*. Furthermore, we observed that PfCaM and PfHint-1 overexpression led to an altered profile in calcium-sensitive proteolysis that correlates with the changes in calcium homeostasis. Taken together, our results show the involvement of both PfCaM and PfHint-1 in calcium homeostasis and calcium-regulated proteolysis in *Plasmodium falciparum*.

Supported by:FAPESP, CAPES, CNPq
Keywords: Calmodulin; histidine triad protein; cell signalling

PV018 - **EXPRESSION OF CALPAIN-LIKE PROTEINS AND EFFECTS OF CALPAIN INHIBITOR MDL28170 ON *STRIGOMONAS CULICIS* WILD TYPE AND APOSYMBIOTIC STRAINS**

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Members of the Trypanosomatidae family are uniflagellated protozoa classified into heteroxenics and monoxenous, depending if their life cycle alternate or not among an insect vector and a vertebrate host (or plant). Although monoxenous parasites constitute the largest segment in the family, they are still overlooked. Interestingly, in this group, there are species that harbor symbionts in their cytoplasm, such as *Strigomonas culicis*. The presence of the symbiont induces changes in the morphology and cellular processes of the trypanosomatid, including an alteration in peptidases expression. Considering the critical roles played by these enzymes in the parasite life cycle, proteolytic inhibitors could be interesting compounds for an alternative chemotherapy. Moreover, analysis of the available sequenced genomes of some trypanosomatids indicate that among the most abundant peptidases are the calpain-like proteins, which are particularly abundant in the genome of species harboring symbionts. In this context, the goal of this work is evaluate the differential expression of the calpains of *S. culicis* and the effects of the calpain inhibitor MDL28170 on the growth rate of both strains. An increased expression of calpain homologues was detected in the aposymbiotic strain, in comparison to the wild strain, by flow cytometry. Moreover, the calpain inhibitor MDL28170 caused a dose-dependent reduction in the proliferation rate of both strains. Also, MDL28170 was able to modulate the expression of calpains homologues in the aposymbiotic strain. Further studies to better characterize calpains in other trypanosomatids are in progress, aiming to unveil calpain function in these protozoa and add new possibilities for the exploitation of calpain inhibitors as an alternative treatment for diseases caused by trypanosomatids.

Supported by:MCT/CNPq, FAPERJ, CAPES, FIOCRUZ
Keywords: Strigomonas culicis; calpain; mdl28170

PV019 - **SUSCEPTIBILITY OF *LEISHMANIA MAJOR* TO ATM AND ATR INHIBITORS SUGGESTS THE EXISTENCE OF PIKK SIGNALING DEPENDENT CELL CYCLE AND REPLICATION CONTROL PATHWAYS**

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Countless metabolic pathways are present in living cells, ranging from those involved in compound biosynthesis and energy production, to those that take place in cell proliferation or death. Monitoring and refined control of all these processes are performed by cell signaling pathways, whose core proteins make up the family of phosphatidylinositol 3-kinase-related kinases (PIKKs). Ataxia Telangiectasia Mutated (ATM) and Ataxia Telangiectasia and Rad3 related (ATR) are two important PIKK proteins that act in response to DNA damage, phosphorylating a great number of proteins to control genomic integrity. The genus *Leishmania* belongs to a group of early divergent eukaryotes in evolution and presents a highly plastic genome, indicating the existence of signaling pathways designed to keep the genome integrity of this organism. The objective of this work was therefore to demonstrate that the use of specific inhibitors for ATR and ATM in *Leishmania major* alters their normal growth patterns in culture, cell cycle and morphology. Bioinformatics analyzes revealed the existence of the putative PIKK genes ATR and ATM, in addition to mTOR and DNA-PKcs. Molecular modeling and docking tools have made it possible to verify that inhibitors VE-821 and KU-55933 have binding affinity for the catalytic site of ATR and ATM, respectively. Promastigotes of *L. major* exposed to these inhibitors show growth impairment, which is clearer during the first 24 hours, and is compatible with the accumulation of cells in the G_1 stage as assessed by flow cytometry. Morphological alterations were also found, such as the presence of cells lacking kinetoplast or nucleus, as evaluated by fluorescence microscopy. These results suggest that the use of specific inhibitors of ATR and ATM in *Leishmania* interferes in the signaling pathways of this parasite, which may influence both the cell cycle and the maintenance of the integrity of its genome.

Supported by:FAPEMIG
Keywords: Leishmania major; dna damage; cell signaling

PV020 - **CHARACTERIZATION OF *TRYPANOSOMA CRUZI* LIPID BODY FRACTION REVEALS THE CHOLESTEROL PATHWAY AFTER STORAGE IN THE RESERVOSOMES**

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Trypanosoma cruzi epimastigotes are the developmental forms responsible for parasite proliferation and survival in the digestive tract of the insect vector. Lipids from vector diet reach the endocytic apparatus and are stored inside reservosomes, to be used according to epimastigote needs (Pereira et al, PLoS One 10 e0128949, 2015). Parasite must deal two lipid-storing compartments: reservosomes and lipid bodies (LBs), and this prompted us to explore the connection between the two organelles in lipid traffic. In this work, we also observed by electron microscopy a close relationship between LBs and endoplasmic reticulum, mitochondria and glycosomes (peroxisome like compartments). In higher eukaryotes, these organelles represent important metabolic routes for lipids. Moreover, we developed a reproducible protocol to isolate epimastigote LBs. They are surrounded by a phospholipid monolayer formed mainly by PC, PE and PI. Exogenous cholesterol taken up by endocytosis is stored in ester form and represent almost 90% of total lipids. We did not detect ergosterol in LB fraction. Triacylglycerol, diacylglycerol, monoacylglycerol and free fatty acids were found and represent 10-15% of neutral lipids. Some long chain fatty acids, such as dihomo-γ-linolenic acid (C20:3), docosahexaenoic acid (C22:6), arachidonic acid (C24:0) were found in lower proportions and could be in involved in signaling pathways. The uptake of exogenous cholesterol by endocytosis and its storage in the ester form lead us to conclude that there is a connection between reservosome's cholesterol and LBs. Cholesterol should be temporarily stored in reservosomes before being esterified by an ACAT-related enzyme in lipid bodies. **Supported by:**Capes
Keywords: Lipid transport; lipid storage; cellular cholesterol

PV021 - **NEW INSIGHTS OF APICOPLAST GENOME REPLICATION AND SEGREGATION IN *TOXOPLASMA GONDII***

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Toxoplasma gondii harbors a non-photosynthetic secondary plastid (apicoplast), which contains a 35kb circular genome. The maintenance of the genome is essential for apicoplast function and for parasite survival. Thus, proteins involved in the apicoplast genome replication are potentially drug targets for treating toxoplasmosis. In this work we characterized three putative proteins predicted to be involved in the apicoplast genome replication: DNA polymerase I (Prex), single stranded DNA binding protein (SSB) and DNA gyrase domains A and B. Construction of endogenous tagged parasites for DNA polymerase, SSB and Gyrase A and posterior analysis by structure illumination microscopy (SIM) confirmed the apicoplast localization of these proteins and also showed their association to the apicoplast nucleoid. Construction of inducible mutants for DNA Gyrase domains A and B and Prex showed that these proteins are essentials for apicoplast genome replication and parasite viability. Knockdown of Prex and DNA Gyrase domains A and B caused a remarkable decrease in the apicoplast genome copy number and also caused apicoplast loss. On the other hand, SSB is not essential and did not affect parasite viability. These results validate Prex and DNA gyrase as drug targets in *T. gondii*. Additional analysis of tagged parasites by SIM also provided information regarding the dynamic of apicoplast genome segregation in *T. gondii*. Observations of apicoplast nucleoid along the parasite division showed that the nucleoid has different morphologies according to parasite cell cycle. While a single dot nucleoid predominates during parasite G1 phase, dumbbell or beaded shape nucleoids are commonly seen during S phase. However, during mitosis/cytokinesis phase most apicoplasts contains divided nucleoids. This suggests that the apicoplast nucleoid partition and segregation occur during the S phase and finish during early mitosis of parasite cell cycle. **Supported by:**Faperj, CNPq, Capes, NIH
Keywords: Prex; nucleoid; dna gyrase

PV022 - **ASSESSMENT OF CRISPR-CAS9 GENOME EDITING PROTOCOLS FOR FAST GENERATION OF *TRYPANOSOMA CRUZI* KNOCKOUT PARASITE CELL LINES**

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Genome manipulation of the protozoa *Trypanosoma cruzi* is hampered by the lack of efficient tools since a functional RNAi pathway is absent in this parasite and knockout strategies using conventional homologous recombination (HR) protocols cannot be used for the study of the large number of multi-gene families. Although we have showed that Zinc Finger Nucleases (ZFNs) can improve gene knockout efficiency in *T. cruzi*, the high cost of ZFNs design and synthesis poses limits to its widespread applications. Recently, two groups have demonstrated that CRISPR/Cas9 system can be used to perform genome editing in *T. cruzi*. In these reports, constitutive expression of Cas9 was used and microhomology mediated end joining (MMEJ) or HR mechanisms were described to take place as DNA repair mechanisms, resulting in efficient gene knockout or endogenous gene tagging. Because there are concerns regarding off target effects that might result from constitutive expression of Cas9, we tested different CRISPR/Cas9 protocols, using the *gp72* gene as a target sequence. Parasites knockouts to *gp72* gene can be easily recognized due to its detached flagellum phenotype. Transfection of *T. cruzi* CL Brener epimastigotes stably expressing Streptococcus pyogenes Cas9 (SpCas9) with in vitro transcribed single guide RNA (sgRNA) and a single strand oligonucleotide donor sequence (ODS) with stop codons, flanked by 40 nucleotide *gp72* homologous sequences, results in efficient *gp72* gene knockout, 3 days after transfection. Remarkably, in the absence of the ODS, transfected parasites showed an aberrant morphology, with only few cells displaying the typical flagellum detachment phenotype. Epimastigotes transfections with recombinant Cas9 from Staphylococcus aureus (SaCas9) associated with *in vitro* transcribed sgRNA were able to generate *gp72* knockout mutants only in the presence of the ODS, further indicating that the parasite favours the HR DNA repair pathway. **Supported by:**Cnpq
Keywords: Gp72; crispr/cas9; recombinant cas9

PV023 - **THE ROLE OF THE CYTOSTOME-CYTOPHARYNX COMPLEX IN THE ENDOCYTIC ABILITY OF INTRACELLULAR AND EXTRACELLULAR AMASTIGOTES OF *TRYPANOSOMA CRUZI***

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Trypanosoma cruzi is a protozoan parasite that causes Chagas' disease. During its life cycle, the parasite presents two proliferative forms: epimastigotes are extracellular forms, present in the midgut of the insect vector; amastigotes are intracellular forms, present in the cytoplasm of vertebrate host cells. In epimastigotes, endocytosis is well characterized and occurs through a specialized membrane domain called cytostome-cytopharynx complex. Amastigotes also possesses a cytostome-cytopharynx complex, however the participation on endocytosis of these forms have not been elucidated. Extracellular amastigotes can be obtained from the supernatant of infected cells and are capable to establish and maintain infection in vivo and in vitro. It has been shown that intracellular amastigotes and extracellular amastigotes have differences in their surface proteins. In this work, we analyzed and compared the ultrastructure of the cytostome-cytopharynx complex of intracellular amastigotes, within their host cells, and extracellular amastigotes, obtained from the supernatant of infected LLC-MK2 cells, using high-resolution electron microscopy technics, such as electron tomography and FIB-SEM. We also compared the endocytic ability of intracellular amastigotes, obtained through cell lysis, with that of extracellular amastigotes. We observed that intracellular amastigotes have a cytostome-cytopharynx complex similar to that of epimastigotes. However, after isolation through host cell lysis, those amastigotes undergo ultrastructural modifications in the cytostome-cytopharynx complex, such as enlargement and microtubule mispositioning, leading to an impairment of the endocytic ability. Extracellular amastigotes from the supernatant do not possess a cytostome-cytopharynx complex neither the ability to uptake transferrin from the culture medium. Those observations are new evidences of structural and functional differences between intra and extracellular amastigotes. **Supported by:**CNPq
Keywords: Trypanosoma cruzi; amastigotes; endocytosis

PV024 - **THE DYNAMIC CYCLE OF MRNAS IN TRYPANOSOMA CRUZI.**

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Trypanosomatids present biological particularities, such as the absence of canonical promoters for RNA polymerase II and polycistronic transcription, showing that the regulation of the gene expression occurs by post-transcriptional events. Among these events, the control of mRNA degradation and mRNAs access to the translation machinery are directly related to the adaptation during their development. In eukaryotes, mRNAs that are not being translated, or those directed for degradation, are compartmentalized into distinct cytoplasmic structures termed "mRNP Granules". These granules are classified in P-bodies and stress granules (SG) and play a key role in the post-transcriptional regulation of gene expression. These structures can interact in a dynamic cycle with the exchange of mRNAs among translation, storage and degradation, indicating their decisive role in the control of gene expression. In *T. cruzi*, we observed that TcDHH1 and TcXRNA (P-bodies and SG markers in eukaryotes) have granular cytoplasmic localization, are constitutively expressed throughout metacyclogenesis, accumulate around the nucleus and colocalized partially, evidencing the presence of distinct granules. From these results, we investigated the dynamic movement of mRNAs among the storage, degradation and translation machineries in *T. cruzi*. RNA sequencing analysis of poly(A)+ RNA population showed that 2% of genes are differentially expressed comparing epimastigotes and epimastigotes under nutritional stress. However, ribosome footprints mapping showed that although almost all of the transcripts are present in both conditions, only 45% of the translated transcripts are common between them. In addition, TcXRNA and TcDHH1 granules do not share mRNAs, suggesting that these granules have distinct functions. TcXRNA granules share some mRNAs with the translation machinery in stressed parasites, while TcDHH1 does not, suggesting that the transcripts associated to TcDHH1 are being degraded.
Supported by:CAPES, FIOCRUZ and Fundação Araucária.
Keywords: Mrnp granules; translation; gene expression regulation

PV025 - **POTENTIAL INFECTION OF *STOMOXYS CALCITRANS* BY *LEISHMANIA SP.***

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Stomoxys calcitrans, stable fly, is a Diptera of great veterinary importance. Mechanical vector of several pathogens, this insect leads to considerable economic losses in the livestock sector. Both males and females flies are hematophagous and present a persistent and aggressive feeding behavior, which favors the transmission of pathogens to their hosts, mainly cattle. Among the parasites mechanically transmitted by *S. calcitrans* are *Leishmania* species, protozoans that cause leishmaniasis, a group of diseases which affects humans and animals. Based on these information, the objective of the work is to investigate the interaction between *S. calcitrans* and *Leishmania* species. For *in vitro* assays, explanted guts were allowed to interact with *Leishmania amazonensis* ($1x10^6$) for 1h and then washed and homogenized and the parasites that remained bound counted. It was obtained a mean of $2.7x10^3$ parasites per gut. For *in vivo* assays, guts of flies fed with bovine blood containing $1x10^7$ *Leishmania infantum* per mL were dissected 30 minutes post feeding, presenting a mean number of $5.6x10^3$ viable parasites per gut. These preliminary results indicate a putative interaction between these organisms, once *L. amazonensis* was able to bind to the intestinal epithelium of *S. calcitrans*, besides *L. infantum* remaining viable in the intestine of *S. calcitrans* after feeding. The next steps will be investigating for how long the parasite remains viable in the fly gut and the possibility of flies fed with infected blood to transmit the parasite to mice under laboratory conditions. **Supported by:**CAPES
Keywords: Stomoxys calcitrans; leishmania amazonensis; leishmania infantum

PV026 - **FATTY ACID METABOLISM IN *TRYPANOSOMA CRUZI*: LIPIDS AS AN ENERGY SOURCE AND CHARACTERIZATION OF AN ACYL-COA DEHYDROGENASE**

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T. cruzi, along its life cycle, is submitted to different environmental conditions in which different carbon and energy sources, such as carbohydrates, amino acids and lipids, are available. A body of evidence exists this parasite can metabolize carbohydrates and amino acids for ATP production. However, little is known about the fatty acid metabolism and its importance for parasite bioenergetics. The beta-oxidation of fatty acids consists of four steps that render $FADH_2$, NADH, acetyl-CoA and a fatty acid two carbon unit shorter that the initial one. The first enzymatic step consists in a dehydrogenation, performed by enzymes belonging to the acyl-CoA dehydrogenase (ACAD) family. This enzymatic step transfers electrons to FAD and then to the ubiquinone pool, feeding OxPhos. In this work, we report that *T. cruzi* is able to uptake and metabolize fatty acids, ensuring its viability and resistance in nutritional stress conditions. *T. cruzi* genome encodes a single putative acyl-CoA dehydrogenase. We successfully expressed this enzyme in *Escherichia coli* BL21 codon plus (DE3) and kinetic results were obtained from its activity measurements (Km=14.4 + 1.125 μM, Vmax=0.024 + 0.0005μmols/min.mg^{-1} protein when using isobutiryl-CoA as the substrate, and Km=1.469 + 0.28 μM, Vmax=0.011 + 0.0005 when using palmitoyl-CoA as the substrate). Also, kinetic data were obtained from cell-free extracts of epimastigotes for isobutiryl-CoA (Km=51.08 + 8.585 μM, Vmax=3.052 + 0.1916), lauroyl-CoA (Km=37.03 + 6.725 μM, Vmax=2.979 + 0.1879), palmitoyl-CoA (Km=47.95 + 14.07 μM, Vmax=2.554 + 0.2295) and estearoyl-CoA (Km=60.94 + 26.18 μM, Vmax=2.298 + 0.3520 μmols/min.mg^{-1} protein). The recombinant enzyme had preference for FAD as cofactor but it was active when $NAD(P)^+$ was offered as co-factor. Preliminary assays indicate it is a mitochondrial enzyme. The participation of this enzyme in beta-oxidation and bioenergetics is now being investigated. **Supported by:**CNPq
Keywords: Fatty acid; beta-oxidation; acad

PV027 - **THE KINETOPLAST OF TRYPANOSOMATID PROTOZOA: ULTRASTRUCTURE AND KDNA REPLICATION**

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Trypanosomatids contain unusual structures, as the kinetoplast, the portion of the single mitochondria that contains a unique array of circular and interlocked DNA, known as kDNA. Such network is composed by dozens of maxicircles and hundreds minicircles, whose replication is tightly controlled during the cell cycle and involves a large repertoire of proteins. Two different mechanisms are used during kDNA replication: polar mode, where the replicated minicircles are attached to network at the antipodal site; and the ring mode, where the kDNA disk rotates in relation to the antipodal site and the new minicircles are attached uniformly all over the network. In trypanosomatids, kDNA arrangement varies depending on the species or even along the life cycle. During Trypanosoma cruzi metacyclogenesis, epimastigotes differentiate into trypomastigotes. Here, our results obtained by electron microscopy, showed that the disk-shaped kinetoplast present in the replicative form only acquires a globular format after the repositioning of this structure toward the posterior end of the cell body, when the condensed kDNA fibers becomes more relaxed. Furthermore, data obtained by atomic force showed a more uniform distribution of kDNA molecules in epimastigotes when compared to intermediate forms and trypomastigotes, which contain kDNA fibers more concentrated in some regions of the network. In symbiont-harboring species, the kinetoplast presents atypical shapes (bow or trapezoid format) and kDNA fibers are loosely arranged in the network, as revealed by electron tomography. In this work we also investigated the kDNA replication using fluorescent nucleotide analogs. Preliminary results indicate that this process only occurs on epimastigotes, but not on intermediate or trypomastigote forms. Kinetoplast antipodal sites seem to be present in the kinetoplast of symbiont-bearing trypanosomatids. **Supported by:**FAPERJ AND CNPq
Keywords: Trypanosomatid; kinetoplast; kdna

PV028 - **GENE DISRUPTION OF BETA- AND DELTA-AMASTINS FROM *TRYPANOSOMA CRUZI* BY CONVENTIONAL KNOCK OUT AND CRISPR-CAS9 EDITING**

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The amastins are membrane proteins that are differentially expressed during *T. cruzi* life cycle, and it has been shown in *T. cruzi* and *Leishmania* that they can be associated to invasion, parasite survival, or multiplication in their hosts. In silico analysis allowed amastin classification in α, β, γ and δ subfamilies in Trypanosomatids, and we identified α, β and δ-amastins in *T. cruzi*. In this work, we would like to improve the functional characterization of amastins, through β-amastins deletion by homologous recombination and δ-amastins by using the CRISPR/Cas9 system. Towards β-amastins deletion, NeoR and HygroR cassettes were constructed and transfected into CL Brener to knock out these genes by the homologous recombination. The integration of the two deletion cassettes into β-amastins locus were confirmed by PCR, however the phenotypic analyses have shown no changes in metacyclogenesis, but additional experiments are underway. To characterize δ-amastins, which are encoded by a multi copy family highly expressed in amastigote forms, we decided to use the CRISPR/Cas9 system to disrupt these genes. To test this system, sgRNA was designed to target ever single δ-amastin gene using EuPaGDT. After that, G epimastigotes expressing δ-amastin fused to GFP were co-transfected with *Staphylococcus aureus* Cas9 recombinant protein, *in vitro* transcribed sgRNA targeting δ-amastin and an oligonucleotide donor sequence containing stop codons flanked by homology arms. One week after transfection, the δ-amastin editing was checked by flow cytometry and RFLP-PCR. The flow cytometry analysis showed specific and strong reduction of GFP fluorescence (close to 100%) in δ-amastin::GFP parasites. The PCR analysis also showed that besides the ectopic copy, the endogenous copies were edited. Up to now, similar approaches are being carried to disrupt amastins in other strains such as Y and CL Brener.
Supported by: Capes, CNPq, Fundação Araucária **Keywords:**Amastin; crispr/cas9; knock out

PV029 - **CONSERVED MOTIFS IN NUCLEAR GENES ENCODING PREDICTED MITOCHONDRIAL PROTEINS IN *TRYPANOSOMA CRUZI***

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Trypanosoma cruzi, the protozoan parasite that causes Chagas' disease, exhibits peculiar features that make it a good model to study post-transcriptional regulation. The presence of a unique mitochondrion in the kinetoplastid protozoans is remarkable. In spite of the fact that the mitochondrial DNA constitutes up to 25% of total cellular DNA, the structure and functionality of the mitochondrion are absolutely dependent on the expression of the nuclear genome. As in other eukaryotes, the mitochondrial localization of nuclear encoded proteins in trypanosomatids is achieved through specific peptide signals. However, there are mitochondrial proteins encoded in the nuclear genome that lack of a peptide signal. Alternative, protein targeting to subcellular organelles via mRNA localization has been also recognized and specific mRNA localization towards the mitochondria has been described in other eukaryotes. Seeking for mitochondrial localization signals in *T. cruzi*, we built a database of nuclear genes encoding predicted mitochondrial proteins, which was named MiNT. A conserved peptide signal, M(L/F/R)(R/S/T/A)(R/S)SSRR, named TryM-TaPe was found in 54% of the database. In addition, the search for compositional signals in the transcripts of MiNT enabled the identification of a conserved element, RDAARRD, amongst others. Since the latter motif is recognized by the *T. brucei* TRRM3 protein, which is enriched in mitochondrial membrane fractions, a putative zipcode role is suggested for this element. Globally, we provide an inventory of the mitochondrial component proteins in *T. cruzi*, and found out conserved peptide and nucleotide motifs that may constitute mitochondrial localization signals.

Supported by:CSIC & PEDECIBA
Keywords: Mitochondria; mrna localization; trypanosoma cruzi

PV030 - **GENE EXPRESSION REMODELING ALONG THE TRYPANOSOMA CRUZI CELL CYCLE.**

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The mechanism governing gene expression regulation along the proliferative cycle of *Trypanosoma cruzi* are still poorly understood. In view of the biological and therapeutic relevance of the parasite replication we sought to apply RNA-seq approaches to identify global gene expression patterns during the progression of the parasite cell cycle. For that purpose, we deep sequenced the polyA-RNAs (transcriptome) and the ribosome footprints (translatome) of different cell cycle phases. We analyzed epimastigotes of a TcI strain synchronized with hydroxyurea, obtaining cell populations in G1, S and G2/M phases (at 70% enrichment). We extracted RNA and prepared RNA-seq libraries for NGS. We found 305 differentially expressed mRNAs (DEGs) (fold change ≥1.5, p-value <0.01) in the total RNA fraction. These transcriptomic changes involve proteins dedicated to carbohydrates metabolism and energy production at G1-phase (70 genes), DNA and chromatin replication at S-phase (97 genes) and microtubules-based movement at G2/M-phase (138 genes). For the ribosome profiling, we only studied the G1- S transition. Interestingly, translational regulation affects more than 1150 genes at >2.5-fold change, 20% and 80% of which were up-regulated in G1 and S-phase respectively. Enriched molecular functions in the latter dataset include ribosome synthesis, nucleotide metabolism and microtubule dynamics. DEGs identified in transcriptome and translatome have distinctive structural and functional properties, such as distance to the transcription start site, GC content, codon usage adaptation, gene and untranslated region (UTRs) length. We found specific sequence and structural RNA motifs in the UTRs, including the known CS sequence responsible for the periodical expression of some mRNAs. Several known as well as novel RNA binding proteins are found to periodically modify the abundance of their mRNA; indeed, some are predicted to bind the enriched RNA sequence motifs.

Supported by:CSIC, PEDECIBA, ANII
Keywords: Rna-seq; cell cycle; ribosome profiling

PV031 - **MICROBICIDAL EFFECT OF *TITYUS SERRULATUS* VENOM IN *TOXOPLASMA GONDII* INFECTION: IDENTIFICATION OF THE KEYS MOLECULES**

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INTRODUCTION: *Toxoplasma gondii* (Tg), is able of subverting the immune system, persisting in various organs of their hosts. Inflammatory mediators (IM) produced during infection by Tg are crucial to control of parasite, avoiding the disease reactivation. We demonstrated that venom of yellow scorpion *Tityus serrulatus* (TsV) is able to induce production of IM by Tg-infected-macrophages (MΦ), reducing parasite load. Thus, is important the characterization of effector molecules. METHODS AND RESULTS: TsV was separated in 7 fractions (Ts1-Ts7)(Gel Filtration). Only fractions Ts6 and Ts7 presented activity, inducing IM and toxoplasmicidal action by MΦ. To isolate effector molecules, subfractions of Ts6 fraction was obtained (Cation Exchange Chromatography). MΦ were plated, infected and stimulated with the Ts6 subfractions. Parasite replication assay was done counting the number of intracellular Tg after 48h infection/stimulus. The subfraction Sub6-C showed ability to control Tg growth, resulting lower parasite load, compared with non-stimulated MΦ and others subfraction. Sub6-C was characterized, and by bioinformatics were generated three peptides from the original sequence of the Sub6-C (Sub6-C1,C2a,C2b). MΦ were plated, infected (above) and stimulated with Sub6-C1,2a,2b[100,50,25ug/ml].To in vivo assay, B6 mice were infected with 20 cysts and treated(Sub6-C2a,2b) 0-7 day pos infection (dpi) at 1mg/Kg/d. Number of brain cysts was counted in 30 dpi. We show that Tg-stimulated-MΦ Sub6-C1/C2a had lower parasitic burden, compared with Tg-non-stimulated-MΦ at 48h. In contrast, Tg-stimulated-MΦ Sub-C2b has greater intracellular growth of Tg. *In vivo*, the results was similar, where Tg-treated-mice C2a, have less cysts when compared with control, and Tg-treated-mice C2b have similar brain cysts. CONCLUSION: Our data has shown toxoplasmicidal activity of TsV and its components, being a promising “toll” for design an efficient drug inducing control of Tg replication.
Supported by:CNPq/FAPEMIG
Keywords: Immunoregulation; immunoparasitology; toxinology

PV032 - **GENERATION OF *LEISHMANIA INFANTUM* DEFECTIVE IN THE BIOSYNTHESIS OF REPEATING DISACCHARIDE PHOSPHATE UNITS (LPG2 KNOCKOUT)**

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Introduction: We have recently explore LPG1-deficient mutant of *L. infantum* to demonstrate that LPG absence impairs the activation of MAPK signaling pathway in infected macrophages. However, in amastigotes the dominant repeating disaccharide phosphate units of LPG are shared by secreted proteins (PPGs, PGs e sAP), which are described to present immunomodulatory effects on macrophage function. Aiming to assess the role of LPG and these other molecules bearing disaccharide-phosphate repeating units, herein we describe the gene disruption of the Golgi GDP-Man transporter gene (*lpg2*) in *L. infantum*. Methods: The parasite knockout for *lpg2* was obtained by gene targeting deletion through homologous recombination. The 5’UTR and 3’UTR sequences flaking the *lpg2* gene from *L. infantum* Ba262 were amplified by PCR from genomic DNA. Then cloned flaking the Neo and Hyg markers. Promastigotes in early log-phase were electroporated using the high voltage. Results: The cassettes containing the flaking regions and antibiotic markers were all sequenced for correct assembly confirmation. After the disruption of the first allele, the heterozygous (*lpg2*$^{+/-}$) parasites were expanded in the presence of G418. After the disruption of the second allele, the parasites *lpg2*$^{-/-}$ were expanded in the presence of Hygromycin B and G418. Lpg2$^{-/-}$ parasites showed a delayed replication compared to the wild type but reached late-phase in axenic cultures. The presence of markers was confirmed by PCR. However, it was possible to amplify the coding sequence of *lpg2* in the predicted homozygous (*lpg2*$^{-/-}$), raising questions about the copy number of this gene in the *L. infantum* genome. Southern-blot assays are underway to address this question. Conclusion: Data obtained here demonstrated that the *lpg2* gene was partially disrupted. Moreover, *lpg2* gene could be present as a multi-copy gene in *L. infantum* genome, supporting the use of CRISPR system.
Supported by: FAPESB, IGM, Fiocruz-BA
Keywords: Leishmania; knockout; phosophoglycans

PV033 - **EPIDEMIOLOGY OF CUTANEOUS LEISHMANIASIS IN ACRE STATE: STUDY ON THE SANDY FLY FAUNA, BLOOD MEAL PREFERENCE AND CHARACTERIZATION OF CIRCULATING *LEISHMANIA* SPP.**

PEREIRA, T.A.[*1]; PEREIRA, D.P.[1]; BOITÉ, M.[1]; SILVA, F.S.[1]; DA COSTA REGO, T.A.N.[1]; PINTO, I.S.[1]; MOREIRA, R.B.[1]; RODRIGUES, A.F.[1]; OLIVEIRA, M.[1]; BRAZIL, R.P.[1]; BRITTO, C.[1]
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We propose to investigate the heterogeneity of the sandfly fauna in the municipality of *BRAZIL*éia, to bring more insights concerning the epidemiology of cutaneous leishmaniasis (CL) in Acre State, Brazil. Following species identification, we evaluated the infection rate by *Leishman*ia in the vectors, their blood sources and correlate the parasite species found in insects with those identified in lesions of patients living in the same region. Sandflies were captured from September 2013 to January 2015 in seven localities from rural areas of *BRAZIL*éia. Parasite detection in insects was performed individually using a multiplex PCR targeting both, the *Leishmania* kDNA and a sandfly gene (cacophony). *Leishmania* sp. was identified by PCR directed to the hsp70 gene and sequencing. A sampling of 4,477 sandflies being 2,176 males, 2,203 non-blood-fed females and 98 blood-fed females was collected. So far, 15 sandfly genera and 64 species were morphologically identified. The search for *Leishmania* DNA was conducted in 866 non-blood-fed females, reaching a positivity of 3.12% (27/866) with the identification of *L. (V.) braziliensis*, *L. (V.) guyanensis* and *L. (V.) braziliensis/peruviana*. Analysis of blood meal source was carried out by cyt b sequencing in 98 blood-fed females, resulting in the finding of 15 vertebrate species. In 8 out of 98 fed females (8.2%), it was possible to verify the presence of L*. (V.) shawi, L. (V.) guyanensis, L. (V.) braziliensis, L. (V.) braziliensis/peruviana* and *Endotrypanu*m sp DNAs. Skin biopsy imprints or biopsy fragments from 18 out of 22 individuals with clinical CL suspicious were positive for kDNA-PCR. *L. braziliensis* and *L. guyanensi*s were identified in 61.1% and 5.5% of the positive samples, respectively. For the remaining samples (33.4%), we were not able to identify the parasites to the species level. With the conclusion of this study, we expected to provide new elements for better understanding the CL transmission cycle in the study area. **Supported by:**CNPq, CAPES, FAPERJ, FIOCRUZ **Keywords:**Leishmania; sandflies; acre

PV034 - **MYOSINS EXPRESSION IN *TRYPANOSOMA CRUZI***

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Myosins constitute a motor protein superfamily that interacts with actin filaments and they are involved in many cell processes, including endocytosis. In Trypanosomatids, two myosins – Myosin 1 and Myosin 2 – are conserved in *Trypanosoma brucei, Leishmania spp*. and *Trypanosoma cruzi*. Myosin 1, the most conserved, is included in class If. Myosin 2 is present in Trypanosomatids and *Naegleria* and it is part of the new class XIII characterized by the presence of UBA-like domain. It has been demonstrated that both Myosins 1 and 2 are essential to endocytic events in *T. brucei* bloodstream forms and *Leishmania donovani* promastigotes, respectively. Besides those two myosins, *T. cruzi* CL Brener strain genome revealed an expanded family of myosins with six new members exclusively present in this parasite. The enlarged number of myosins was suggested to be associated to the cytostome-cytopharynx complex, the main endocytic site in *T. cruzi* epimastigote forms. Nevertheless, nothing is known about myosin's expression or function in *T. cruzi*. To better investigate *T. cruzi* myosins, firstly we searched for the eight myosin genes in the Dm28c strain genome. We found homologous for all of them and they are well conserved between those two strains. Afterwards, we searched for the myosin head conserved domain PF00063 in Dm28c genome and found another myosin gene, TcMyo9. Then, we used real time PCR to quantify the nine myosins transcripts in epimastigotes, culture trypomastigotes and intracellular amastigotes of Dm28c strain. In comparison to beta tubulin, all myosins are well expressed on the three developmental forms, specially TcMyo2. TcMyo2 and TcMyo6 transcripts are more abundant in epimastigotes than amastigotes, while TcMyo3 transcripts are more abundant in amastigotes. As our goal is to find myosins related to endocytosis in *T. cruzi*, we will start with TcMyo1 and TcMyo2 to do knockout and localization using the CRISPR-Cas9 system

Supported by:CAPES, CNPq, FAPERJ **Keywords:**Myosin; endocytosis; trypanosoma cruzi

PV035 - **"HEXOSAMINOLYSIS" AS A BIOENERGETIC ALTERNATIVE FOR *TRYPANOSOMA CRUZI* EPIMASTIGOTES**

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T. cruzi uses glucose as carbon and energy source; having a key role in the stress resistance, cell proliferation, invasion and differentiation. Taking into account the glucose uptake, epimastigotes (Epi) have the highest glycolytic flux among all the stages, despite living, in nature, in an environment poor in glucose (Glc). It is also known that the triatomine perimicrovillar membrane (PV0M) is rich in glycoconjugates containing N-Acetyl-Glucosamine (GlcNAc). In addition, microorganisms of the intestinal microbiota degrade components of the PV0M, providing free GlcNAc. Thus, Epi are exposed to GlcNAc in the triatomine intestine. Here we investigated if hexosamines (GlcNAc and its deacetylated form, Glucosamine (GlcN)) can fuel *T. cruzi* metabolism. Our results show that both hexosamines are transported from extracellular medium (GlcNAc $K_m^{app} = 0.058 \pm 0.008$ mM; GlcNAc $V_{max}^{app} = 0.273 \pm 0.053$ nmol per 20 x 10^6 cells / GlcN $K_m^{app} = 0.854 \pm 0.205$ mM; GlcN $V_{max}^{app} = 0.286 \pm 0.024$ nmol per 20 x 10^6 cells). These metabolites are also able to compete with Glc showing that Glc, GlcNAc and GlcN probably share the same facilitated diffusion transport system. Moreover, GlcNAc or GlcN are able to maintain Epi viability and proliferation at the same levels than Glc. We were interested in knowing if hexosamines enter in glycolysis. We evidenced their phosphorylation in Epi cell-free extracts and we found two putative genes in the *T.cruzi* genome (nagA and nagB), possibly deriving hexosamines to Fructose-6-phosphate. Additionally, we demonstrated that hexosamines induce the CO_2 production, indicating that these molecules can be fully oxidized through the TCA cycle and respiratory chain. These metabolites also increased the mitochondrial membrane potential and the O_2 consumption. In addition, both hexosamines stimulated ATP production. Our work is the first report of the bioenergetics role of hexosamines degradation in *T. cruzi* and support the reason for a glycolytic pathway in Epi.

Supported by:CAPES; FAPESP; CNPq **Keywords:**Trypanosoma; metabolism; glucose

PV036 - **RECRUITMENT KINETICS OF HOMOLOGOUS RECOMBINATION PATHWAY IN TRYPANOSOMA BRUCEI AFTER IONIZING RADIATION**

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Trypanosoma brucei has evolved an extracellular lifestyle in the mammalian host mainly using the antigenic variation strategy. The molecular basis for the variation of expressed Variant Surface Glycoproteins involves DNA recombination events resulting of Double Strand Breaks (DSBs) generated from fragile subtelomeric sites. Thus, the mechanisms involved in DSBs generation, as well as molecules working in the DSBs repair processes, seem to have strong correlation with the ability of T. brucei to escape from host's immune system. To ensure genomic integrity eukaryotic cells have evolved two canonical repair pathways in response to DSBs, homologous recombination (HR) and non-homologous end joining. Despite genes involved in HR were found in T. brucei and studies have identified some of the proteins that participate in HR pathway, the recruitment kinetic of the machinery onto DNA during DSBs repair are not clearly elucidated. Using DNA fragmentation and chromatin protein ligation assays we established the recruitment kinetics of HR pathway in response to DSBs generated by Ionizing radiation (IR), involving sequential recruitment of Exo1, RPA and Rad51. The complete process of DSBs took about 5.5 hours evidenced for basal gH2A bound to DNA. We also observed a displacement of RPA from cytoplasm to nucleus as well as a proportion of RPA modified recruited onto DNA in response to DSB. Importantly we found that DSB induced with IR lead to a declining G2/M phases 5–6h after DNA damaging, while an arrest in G1/S phases is maintained. This suggests that HR, which occurs predominantly in late S/G2 phase, solve the DSBs quickly compared to the other DNA damage response pathway that is supposed to be acting on the transition G1/S, for instance the MMEJ. Taken together, these data suggest that the interplay between DNA damage detection and HR machinery recruitment is finely coordinated in T. brucei, allowing these parasites to rapidly repair DNA after DSBs in G2/M proficient phases **Supported by:**FAPESP/CNPQ **Keywords:**Dna damage response; homologous recombination; double strand breaks

PV037 - **THE ALANINE RACEMASE AND THE ALANINE BIOENERGETICS FUNCTIONS IN *TRYPANOSOMA CRUZI***

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L-amino acids have been shown to be relevant in many *Trypanosoma cruzi* biological processes. However, little is known about the functions of D-amino acid metabolism on the biology of the parasite. The proline racemase (PR) was firstly described in *T. cruzi*, as a potent mitogen. PR is also involved in the infectivity of the host cells by the parasite. The occurrence of D-Alanine and an Alanine Racemase (AR) activity was also described in *Leishmania amazonensis*. We identified for the first time in pathogenic protozoa a gene encoding a putative AR (TcAR), a pyridoxal 5'-phosphate (PLP) dependent enzyme that catalyzes the racemization between L-and D-alanine. We cloned and expressed TcAR and characterized its recombinant product (rTcAR) of 43 kDa. Km and Vmax were determined for the reaction in both directions (21.5 ± 6.04 mM and 65 ± 9.4 µmol/min/mg (D to L) and 26.1 ± 7.9 mM and 53 ± 6.3 µmol/min/mg (L to D)), with an optimal pH of 7-10 and an temperature of 25-37°C. Surprisingly, rTcAR was also able to racemize serine with a similar efficiency than alanine (Km : 30.74 ± 11.6 mM and Vmax : 51.2 ± 11.63 µmol/min/mg). Furthermore, contrary to bacterial AR, PLP seems not to be essential for the activity. Immunofluorescence with a specific anti-rTcAR antibody showed a cytoplasmic localization for TcAR. Specific activity was measured along *T. cruzi* life cycle, with higher values in the insect stages when compared to mammalian stages. We demonstrated that, in addition to L-, D-alanine is also involved in osmoregulation. Importantly, only the L isoform can be a donor for transaminases. Interestingly, both D- and L-alanine were able to maintain the parasites viability and intracellular ATP production. Nevertheless, only L-Alanine induced mitochondrial membrane protection and triggered respiration. Taken together our data show a functional TcAR and provide evidence of alanine bioenergetics functions in *T. cruzi*. **Supported by:**CNPq e FAPESP
Keywords:T. cruzi; metabolism; alanine

PV038 - **COMPARATIVE PROTEOMIC ANALYSIS OF LYSINE ACETYLATION IN TRYPANOSOMES**

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Lysine acetylation has emerged as a major post-translational modification regulating diverse cellular processes. The function of this modification has not been extensively explored in protozoan parasites. Here we describe the acetylome of Trypanosoma cruzi epimastigote form and procyclic (PCF) and bloodstream (BSF) stages of Trypanosoma brucei. We detected 389 lysine-acetylated (K-ac) sites in 235 proteins in T. cruzi; 288 K-ac sites in 210 proteins in PCF and 380 K-ac sites in 285 proteins in BSF. Notably, we found distinct acetylation profiles of T. cruzi and T. brucei, with 103 K-ac proteins in common, but only 18 mutual in T. cruzi, PCF and BSF forms. This low number of shared K-ac proteins between the three parasite forms is explained by the fact that PCF and BSF have a very distinct acetylation profile with only 45 K-ac proteins in common. We identified K-ac proteins predicted to be present essentially in all cellular compartments and enriched in several molecular function groups in both parasites, but with distinct profiles between T. cruzi, PCF and BSF. While K-ac proteins from T. cruzi are enriched in oxidation/reduction, such as iron superoxide dismutase A, in T. brucei, K-ac proteins are enriched in metabolic processes. Interesting, in BSF, which use glycolysis as main energy source, presented less acetylation in glycolytic enzymes compared to PCF, which relies on oxidative phosphorylation for ATP production. Also, our proteome analysis identified N-terminal acetylation of different residues in both parasites, a modification involved in regulation of protein degradation and assembly of protein complexes, processes that are important during parasite differentiation. Our results, suggest that protein acetylation is involved in the differential regulation of multiple cellular processes in Trypanosomes, contributing for our understanding of the essential mechanisms for parasite differentiation. **Supported by:**FAPESP and NIH
Keywords: Trypanosomes; acetylation; glycolysis

PV039 - **CHARACTERIZATION OF RNA BINDING PROTEINS THAT ARE DIFFERENTIALLY EXPRESSED DURING THE TRYPANOSOME CRUZI LIFE CYCLE**

VALENTE, B.M.[*1]; TAVARES, T.S.[1]; CAMPOS, C.L.V.[1]; GOES, W.M.[1]; PAIS, F.S.-.[2]; TEIXEIRA, S.M.R.[1]
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Trypanosoma cruzi has three main forms that are biochemically and morphologically distinct and which are programed to rapidly respond to the drastic environmental changes this parasite faces during its life cycle. Unlike other eukaryotes, protein-coding genes in this protozoan are transcribed into polycistronic pre-mRNAs that are processed into mature mRNAs through coupled “trans-splicing” and poly-adenylation reactions. Because of this, control of gene expression relies mainly on post-transcriptional mechanisms that must be mediated by RNA binding proteins (RBP) that control steady-state levels and translation rates of mRNAs. After searching for motifs present in eukaryotic RBPs, we identified in the T. cruzi CL Brener genome, 253 sequences encoding proteins containing RNA recognition motif (RRM), PABP, Alba, Pumillio and Zinc Finger motifs. Using RNA-seq data generated with mRNA present in epimastigotes, trypomastigotes and amastigotes, we analyzed the expression of all T. cruzi RBPs throughout the life cycle of this parasite. Among the genes that are up-regulated in epimastigotes, we identified TcCLB.506739.99, which encodes a RBP containing a zinc finger motif, named TcRBP99. A role of this protein related to parasite differentiation was revealed by the characterization of epimastigotes in which this gene was knocked-out: compared to wild type (WT) epimastigotes, TcRBP99 null mutant showed growth inhibition and reduced capacity to differentiate into metacyclic trypomastigotes. RNA-seq analyses revealed 12 genes that showed reduced expression in TcRBP99 null mutant epimastigotes compared to WT, one of them encoding a protein annotated as protein associated with differentiation, whose mRNA is up-regulated in epimastigotes compared to null mutants. Immunoprecipitation assays showed that TcRBP99 binds to this mRNA, further suggesting a role of TcRBP99 in controlling the expression of proteins that participate in the epimastigote-trypomastigote differentiation.
Supported by:CNPq, FAPEMIG, INCTV **Keywords:**Rna binding protein; rna-seq; gene knockout

PV040 - **CHARACTERIZATION OF AN RNA BINDING PROTEIN DIFFERENTIALLY EXPRESSED IN VIRULENT AND AVIRULENT STRAINS OF TRYPANOSOMA CRUZI TRYPOMASTIGOTES.**

CAMPOS, C.L.V.[*1]; TAVARES, T.S.[1]; OLIVEIRA, A.E.R.[1]; VALENTE, B.M.[1]; SILVA, V.G.[1]; TEIXEIRA, S.M.R.[1]
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Trypanosoma cruzi, the causative agent of Chagas disease, has its genome organized in polycistronic transcription units. This characteristic implies that gene expression regulation post-transcriptional through the action of RNA binding proteins (RBPs), which control the stability, translatability and localization of the mRNA population within the cell. The *T. cruzi* population is highly heterogeneous with strains presenting different biological, biochemical and molecular characteristics. The CL Brener cloned strain highly virulent and cause high parasitemia in animal models of infection. In contrast, the CL-14 clone presents the peculiar characteristic of being non-virulent, displaying no parasitemia or tissue parasitism even when inoculated into immune deficient mice. RNA-seq analyzes demonstrated the RBP encoded by the TcCLB.507611.300 gene has a 3-fold increased expression of in CL-14 trypomastigotes when compared to CL Brener, suggesting that this RBP may have a regulatory role related to the non-virulent phenotype of CL-14. Thus, the present work aims to characterize this RBP by in silico analysis, as well as to evaluate the changes resulting from the increased expression of this gene in CL Brener. Predictions of physicochemical properties as well as subcellular localization of this RBP were performed using the uniprot database. We also determined the presence of the RRM motif, which has been shown to mediate RNA binding and the 3D structure of the protein through homology modeling using I-TASSER. Multiple alignment, ontological analysis and phylogenetic inferences were also performed between the *T. cruzi* sequence and its ortologues in *Trypanosoma spp* and *Leishmania spp* using the MEGA7.0 software. Using the pROCK vector, we transfected CL Brener epimastigotes with an exogenous copy of this gene with the goal of analyzing possible changes in gene expression as well as in the infection capacity of this strain in response to the overexpression of TcCLB.507611.300.
Supported by: Fapemig, INCT **Keywords:**Rbp; in silico analysis; post-transcriptional regulation

PV041 - **METABOLIC ADAPTATION OF *TRYPANOSOMA CRUZI* EPIMASTIGOTES IN RESPONSE TO HYPOXIA**

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Trypanosoma cruzi epimastigotes inhabit hematophagous insect gut, an oxidizing environment that favors parasites proliferation. In addition, anaerobic microbiota inside vector suggests hypoxic events may occur in this compartment. Complementarily hypoxia condition induces an oxidant environment. Therefore, we evaluated hypoxia effects on epimastigotes energetic metabolism. Hypoxic condition was induced by N_2 saturation atmosphere. Thereby, we assessed parasites proliferation cultured in normoxia or hypoxia in absence or in presence of the antioxidant urate for five days. The results showed hypoxia doubled epimastigotes proliferation and urate addition decreased normoxic cells proliferation in 44%. Parasites in hypoxia were more sensitive to urate and its proliferation decreased 79% that indicates the importance of ROS in epimastigotes proliferation under this condition. We also compared ATP content in normoxic and hypoxic cells using CellTiter-Glo® Luminescent Cell Viability Assay kit. Parasites in hypoxia increased ATP levels more than 50%. In order to investigate mitochondrial respiration or glycolysis contribution for ATP production we analyzed epimastigotes growth in hypoxia or normoxia cultured with oligomycin or 2-deoxiglucose. Oligomycin addition in normoxia cells decreased proliferation in 91%, but parasites in hypoxia decreased in 35%. Deoxiglucose treatment decreased normoxic parasites proliferation in 28% and hypoxic cells proliferation diminished 67%. To confirm that parasites in hypoxia uses glycolysis more than mitochondrial respiration we measured mitochondrial complex II-III and IV activity using ferricytochrome C in a spectrophotometry assay. Parasites in hypoxia diminished mitochondrial complex II-III and IV activity in 37% and 67%, respectively. Taken together our results indicates that *T. cruzi* epimastigotes cultured in hypoxia may adapt to use glycolysis as ATP main source which allows parasite to survive and proliferate under hypoxia condition.
Supported by:FAPERJ, CNPq, INCT-EM, IOC/FIOCRUZ
Keywords: Trypanosoma cruzi epimastigotes; ros; hypoxia

PV042 - **MODULAR VARIABILITY OF MULTIGENE FAMILIES ENCODING SURFACE PROTEINS UNCOVERS DIFFERENTIAL DISTRIBUTION OF MOTIFS DURING THE EVOLUTION OF *TRYPANOSOMA CRUZI* DTUS**

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Among the Tritryps, *T. cruzi* owns the largest expansion of multigene families encoding variable surface proteins that are enrolled in host-parasite interactions. The large number of members of these families hinders the assignment of whole genome sequencing reads to a specific gene, as they can align with the same reliability to several loci. Despite their high sequence variability, these genes present motifs (short DNA sequences) that are shared among distinct members. The relative abundance of these conserved motifs can be used to estimate the variability of multigene families among *T. cruzi* strains and DTUs. To this end, we developed a methodology to evaluate the copy number variation of motifs derived from large gene families. This methodology is genome assembly independent and only requires next generation sequence reads and a reference genome. It consists in retrieving all reads that map to any gene of each family, generating all kmers of 30 nucleotides present in these reads and clustering these kmers by sequence similarity to generate conserved motifs. Finally, the deep of coverage of each motif is computed and compared among the evaluated strains. Our methodology was used to estimate the relative abundance of all motifs identified in the MASP, TcMUC and trans-sialidase multigene families in 34 strains from different *T. cruzi* DTUs, revealing differences in their abundance in these parasites. The variation in motifs composition display high phylogenetic resolution, allowing to distinguish the DTUs, and is influenced by the geographic distance among strains from the same DTU. We have also identified motifs from these three gene families that are conserved among all evaluated *T. cruzi* strains, which could constitute promising vaccine candidates and diagnostic targets to Chagas disease. Finally, we evaluated the conservation of these motifs in the *T. cruzi* related parasite *T. rangeli*, providing insights into the evolution of the parasitism in Trypanosomatids. **Supported by:**CNPq, FAPEMIG, CAPES
Keywords: Multigene families; trypanosoma cruzi; copy number variation

PV043 - **WHOLE GENOME SEQUENCING OF *T. CRUZI* FIELD ISOLATES REVEALS EXTENSIVE GENOMIC VARIABILITY AND COMPLEX ANEUPLOIDY PATTERNS WITHIN TCII DTU**

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The taxon *T. cruzi* is divided into six DTUs, named TcI-TcVI. TcII is one of the major DTUs enrolled in human infections in the South America southern cone. Recently, recombination and gene flow within TcII isolates from close geographic regions have been demonstrated based on microsatellites genotyping. However, large scale comparisons of the mitochondrial and nuclear genomes of TcII field isolates have not been performed yet. In the present work, we have sequenced and compared the whole nuclear and mitochondrial genomes of seven TcII strains isolated from chagasic patients in the central and northeastern regions of Minas Gerais, Brazil, revealing an extensive genetic variability within this DTU. Our results indicate that the genomes of the *T. cruzi* isolates analyzed in this study have a contribution of two to five ancestral populations, reinforcing that the evolution of TcII in Minas Gerais has been shaped by recombination events. A comparison of the phylogeny based on the nuclear or mitochondrial genomes revealed that the majority of branches were shared by both markers. The subtle divergences in the branches could be a consequence of mitochondrial introgression events or be caused by the differential evolutionary rate of the nuclear and mitochondrial genomes. The two strains isolated from the central region of Minas Gerais were clustered in the phylogeny analysis and presented a highly similar admixture pattern. These two strains were isolated from the other five by the Espinhaço Mountains, a geographic barrier that could have restricted the traffic of insect vectors during *T.cruzi* evolution in Minas Gerais state. Finally, the presence of aneuploidies was evaluated with the SCoPE methodology, revealing that all the seven TcII strains have a different pattern of chromosomal duplication/loss. This pattern of aneuploidy does not correlate with the nuclear phylogeny suggesting that chromosomal duplication/loss are recent and frequent events in the parasite evolution **Supported by:**CNPq, FAPEMIG, CAPES
Keywords: Genome; trypanosoma cruzi; copy number variation

PV044 - **IS THERE A HISTONE-LINKER IN *TOXOPLASMA GONDII*?**

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In eukaryotes, DNA is packaged with proteins in a complex called chromatin, which is a natural barrier to all DNA-dependent processes, such as transcription. Silenced genes are located in regions of dense chromatin (heterochromatin), while active genes are located in regions of open chromatin (euchromatin). The alternation of those compaction levels is regulated mainly by histones and their post-translational modifications (PTMs). *Toxoplasma gondii* has the four canonical histones (H2A, H2B, H3 and H4), but to date the fifth histone (H1 or histone-linker), has not been identified. In other eukaryotes, H1 links nucleosomes and its absence could interfere with the chromatin condensation in *Toxoplasma*. We identified a hypothetical protein, similar to H1-like of *Kinetoplastidae* that corresponds only to the C-terminal region of a typical H1. In order to evaluate if this protein has histone activity in *Toxoplasma*, we started the characterization by tagging the endogenous protein, which we named TgH1-like. By immunofluorescence assay, we found that TgH1-like is located exclusively in the nucleus. However by Western blot, the protein showed twice the expected size (20 kDa), suggesting the presence of a PTM such as ubiquitination. In addition, we performed a cell fractionation protocol followed by acid extraction of histones, and we observed that TgH1-like precipitates with histone H4. Next, we performed a co-immunoprecipitation assay, that confirmed the interaction between TgH1-like and H4. Although promising, more evidence will be necessary to corroborate the histone function of TgH1-like. We are currently performing gene knockout and immunoprecipitation to identify the protein complex, which may provide more information about the function of this protein in *Toxoplasma*. **Supported by:**ICC-FRIOCRUZ/PR, CAPES, CNPq, Fundação Araucária
Keywords: Toxoplasma gondii; chromatin; histone

PV045 - **INVESTIGATION OF THE PRESENCE OF CATS INFECTED BY *LEISHMANIA INFANTUM* AND ITS IMPORTANCE IN CYCLE TRANSMISSION OF VISCERAL LEISHMANIASIS IN MINAS GERAIS**

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Leishmaniases are diseases caused by flagellate protozoa of the genus *Leishmania* and present a great diversity of clinical features. In Brazil, canine visceral leishmaniasis (CVL) is caused by *L.* (*Leishmania*) *infantum* and is characterized as a severe and zoonotic chronic infectious disease transmitted by *Lutzomyia longipalpis* sand flies. Dogs have been considered the main reservoir; however, the role of cats in the transmission cycle has been discussed. This study aimed to evaluate the presence of naturally infected cats in a CVL enzootic area and their capacity to infect *L. longipalpis*, as well as the presence of these insects in the area. Out of nine cats living in São Joaquim de Bicas, Minas Gerais, Brazil and submitted to cytological and *q*PCR analyses, 5/9 (56%) and 6/9 (66%) respectively, tested positive. The same samples were cultivated in Novy, McNeal and Nicolle (NNN) medium and did not show positivity. All cats involved this study tested negative in xenodiagnosis. One cat tested positive for the feline leukemia virus (FeLV) and feline visceral leishmaniasis (FEVL). The results point to the existence of natural infection in cats living in CVL enzootic areas and to the need for further studies that can establish the role of these animals in the transmission cycle of visceral leishmaniasis in Brazil. **Supported by:**FAPEMIG, CAPES, CNPq, UFMG, HVSA
Keywords: Feline visceral leishmaniasis; lutzomyia longipalpis; xenodiagnosis

PV046 - **FUNCTIONAL CHARACTERIZATION OF PHOSPHOPROTEINS AND DETERMINATION OF THEIR ROLE AS METACYCLOGENESIS MODULATORS IN TRYPANOSOMA CRUZI**

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Protein phosphorylation is a dynamic process that modulates protein function. Analysis of the genomes of three pathogenic trypanosomatids showed that approximately 4% of the genome encode kinases and phosphatases, proteins acting antagonistically in the control of reversible phosphorylation. The aim of this work is to functionally characterize phosphoproteins from *T. cruzi* and to determine the role of specific phosphorylation sites in these proteins as regulators of the differentiation of this parasite to metacyclic forms. These sites showed significantly modulated intensity during in vitro metacyclogenesis in a previous work of our group. Protein coding sequences for each of the genes were cloned in an expression vector designed to express C- and N-terminal GFP fusion proteins in *T. cruzi*. Transfected parasites expressing the proteins were used to determine their subcellular localization by indirect immunofluorescence. Cryomilling, affinity purification and mass spectrometry are being used to determine the interactome for these proteins. Gene knockout will be performed by homologue recombination to determine the participation of these proteins as triggers of the metacyclogenesis process. Site directed mutagenesis of the phosphorylation sites will help to define its role as modulators of the protein function. Analysis by fluorescence microscopy in epimastigote forms of parasites expressing GFP-tagged proteins revealed a strong positive signal at the anterior region to the kinetoplast for the V5BVD3 hypothetic protein and a nuclear signal for the V5BLR6 protein, fluorescence signal for the other proteins was dispersed throughout the cytoplasm. We expect that the results of this work will reveal informations about signaling pathways involved in metacyclogenesis of *T. cruzi*. **Supported by:**CNPq, CAPES
Keywords: Protein phosphorylation; metacyclogenesis; trypanosoma cruzi

PV047 - **DIVERGENT COMPONENTS OF MRNA EXPORT PATHWAYS FROM *TRYPANOSOMA CRUZI* REVEALED THROUGH PROTEOMICS**

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Regulation of gene expression occurs on several levels in eukaryotic organisms and involves transcriptional and post-transcriptional mechanisms. The nucleo-cytoplasmic RNA export is an essential post-transcriptional pathway for gene expression control in eukaryotic cells, but it is still poorly understood in protozoan parasites. Bioinformatic comparisons indicated that few proteins involved mRNA export are conserved in protozoan. Furthermore, there are also significant structural and functional differences between trypanosome and other lineage proteins, strongly suggestive of divergent mechanisms for mRNA export in these parasites. We validated components of the mRNA export pathway in *T. cruzi* previously, as TcSub2 and Hel45. Here, we performed proteomic analysis to uncover proteins associated with TcSub2 and Hel45 in a non-biased manner and with high confidence. The results revealed a significant overlapping of proteins between TcSub2 and Hel45 complexes, indicating an interaction of components. Using a combination of genetic and biochemical approaches we were able to identify conserved and non-conserved components of mRNA export machinery. Interestingly, some of non-conserved components are divergent proteins that are essential for mRNA export, since the knockdown by RNAi in *T. brucei* resulted in mRNA export defect. Altogether, we were able to identify uncharacterized components of the mRNA export machinery that seems to be exclusive of trypanosomes and to obtain mechanistic insights of their function in these important parasites. **Supported by:**Fiocruz, CAPES, CNPq, FAPPR
Keywords: Mrna export; trypanosomes; proteomics

PV048 - **BIOENERGETICS OF THE NUTRITIONAL STRESS IN *TRYPANOSOMA CRUZI* EPIMASTIGOTES**

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The life cycle of *Trypanosoma cruzi* involves the transit of different parasite forms through different environments, determining adaptive biochemical changes. In particular, inside the insect vector, the *T. cruzi* epimastigotes (Epis) must adapt to several adverse conditions, for example temperature and osmolarity changes. As triatomines spend long periods unfed, variations in nutrient availability inside their digestive tube, the territory that Epis colonize, occur. Thus, the survival of the parasite inside these insects depends on their ability to cope with nutritional stress. In this work we investigated the response of Epis to a simple model of severe nutritional stress (SNS). Through cell viability experiments, we observed that Epis present a remarkable resistance to starvation, when compared to phylogenetically related organism, such as *Trypanosoma brucei* procyclic forms. When nutritionally stressed Epis were incubated in LIT medium (To measure recovery capacity after SNS), a shift at the inflection point of the growth curves in parasites from 72h and 96h of stress was observed (2.31 and 4.41 days respectively, when compared to the control), at 120h the parasites were not able to recover. The intracellular concentration of ATP decreased to 85% at 24h and 98% at 72h of treatment. At these time points, autophagy and consumption of lipid droplets were observed. When mitochondrial activity (measured as free Routine activity) was determined, Epis starved for 24h showed significant levels of recovery at 5mM His, than parasites under SNS. Remarkably, under decreased ATP levels, the parasite presented a free Routine activity recovery profile (when recover at 5mM His) even at long periods of NS (72h and 120h) showing that *T. cruzi* is able to restore the mitochondrial activity even under very adverse conditions. **Supported by:**CNPq
Keywords: Trypanosoma; bioenergetics; nutritional stress

PV049 - **CHARACTERIZATION OF *TRYPANOSOMA CRUZI* AND *TRYPANOSOMA RANGELI* CHAP PROTEINS**

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Trypanosomatids have a complex antioxidant system based on the thiol trypanotione, which is synthesized in these organisms by trypanothione synthetase (TS). Although considered a pivotal enzyme on this mechanism, TS is reported to be a single copy gene on the genome. However, *in silico* analyzes of the CL Brener strain genome have identified two other genes annotated as putative TS or TS-like. Missing the synthetase C-terminal domain (pfam03738), these sequences have maintained the amidase N-terminal CHAP domain composed by a cysteine, histidine-dependent amidohydrolase/peptidase (pfam05257), being named as CHAP1 and CHAP2. The aim of this study was characterize the CHAP genes in trypanosomatids using *T. cruzi* and *T. rangeli* as models. Comparative analysis of CHAP1 and CHAP2 predicted proteins across 17 species of trypanosomatids revealed similar sizes of ~250aa and ~650aa, respectively. Except for *Phytomonas* spp., CHAP1 genes were present in all analysed species, revealing mostly mitochondrial localization. In contrast, there is a lack of CHAP2 gene on the *T. brucei*, *T. evansi*, *L. braziliensis*, and *Phytomonas* spp. genomes. Aiming functional studies, the CHAP1 gene from *T. rangeli* (*Tr*CHAP1) and *T. cruzi* (*Tc*CHAP1) were PCR amplified (~715bp), cloned and sequenced. Alignment of the predicted amino acid sequences from *Tc*CHAP1 and *Tr*CHAP1 showed 79% identity, pointing out a conservancy of the CHAP domain despite a few synonymous changes. Recombinant expression of *Tc*CHAP1 and *Tr*CHAP1 in *E. coli* using the pET system was achieved and the ~27kDa proteins were used to generate polyclonal antiserum in Balb/C mice aiming to assess the expression sites and levels of CHAP1 in these species. The possible function of CHAP1 in trypanothione metabolism will be addressed via enzymatic assays using the recombinant proteins. **Supported by:**CNPq, CAPES, FINEP, UFSC.
Keywords: Trypanothione synthetase; antioxidant system; trypanosomatids

PV050 - **MOLECULAR CHARACTERIZATION OF THE *TRYPANOSOMA RANGELI* FLAGELLAR GLYCOPROTEIN 3 (FLA3)**

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Emerging from the flagellar pocket, the trypanosomatids flagellum is attached along the cell body through the Flagellar Attachment Zone (FAZ), a complex composed by 30 proteins in *Trypanosoma brucei*. Among these proteins, the Flagellar Glycoprotein 3 (FLA3) is a stage-specific protein essential for flagellum attachment and cytokinesis. *Tb*FLA3 is exclusively expressed by bloodstream forms, however, a similar protein named *Tb*FLA1BP (~45% identity) is expressed only by procyclic forms. In this study we have identified and characterized a FLA3/FLA1B ortholog in *Trypanosoma rangeli* (*Tr*FLA3). *Tr*FLA3 has 737aa and is ~36% identical do FLA3 and FLA1BP, being expressed by both epimastigotes and trypomastigotes. Although *Tr*FLA3 lacks the N-terminal transmembrane region observed in *Tb*FLA3, the N-terminal signal-peptide as well as the transmembrane and intracellular portions located at the C-terminus was preserved. A polyclonal antiserum anti-*Tr*FLA3 have recognized proteins of ~100 and ~250kDa in total *T. rangeli* extracts, which is higher than the expected molecular weight for *Tr*FLA3 (~80kDa) and could be due post-translational glycosylation as reported for *Tb*FLA3. Preliminary IFA assays indicate that *Tr*FLA3 co-localizes along the flagellum as observed for *Tb*FLA3 and *Tb*FLA1BP in *T. brucei*. Due both sequence and localization similarity, we hypothesized that *Tr*FLA3 is involved on the parasite FAZ, however, biological significance of the observed differences remains to be addressed. **Supported by:**CNPq, CAPES, FINEP and UFSC **Keywords:**Flagellum; faz; fla1bp

PV051 - **MICROBIOTA OF THE TRIATOMINE SALIVARY GLANDS AND IT´S RELATION WITH ANTI-HEMOSTATIC PROPERTIES**

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Chagas´disease is caused by the parasite Trypanosoma cruzi, and is transmitted through triatomines (Hemiptera: Reduviidae). . In the last year, many studies of triatomine gut microbiota has gained relevance it´s potential role to modulate vector competence. However, little is known about the microbiota present in the salivary glands of the triatomines. In this work cultivable and non-cultivable salivary glands bacteria were investigated by colony forming unit (CFU) assays and by sequencing 16S rRNA gene. Our results demonstrate that the salivary glands of the triatomine have different cultivable bacteria. In some species of genus *Rhodnius,* we observed the *Rhodococcus rhodnii* in salivary glands and species *Triatoma infestans, Panstrongylus megistus* and *Triatoma BRAZILiensis* were found *Proteus mirabilis, Enterococcus faecalis* and *Williamsia seridenes*, respectively. However, some genera of non-cultivable bacteria appear to be specific to certain triatomine hosts like Arsenophonus to Triatoma and *Enterobacteriaceae* to Rhodnius, supporting the hypothesis that of specificity between bacterium and triatomine. Furthermore, we investigated if the bacterium could influence in the salivary gland anti-hemostatic properties. For this, hemostasis assays were performed on treated and untreated *T. infestans* salivary glands with antibiotics. It was verified that in the untreated samples the plasma coagulation took longer than treated salivary glands, suggesting that the bacteria could be influencing the blood coagulation pathway. This study was the first investigate about the microbiota in the salivary glands in triatomine and we believe that these bacteria may contribute with anti-hemostatic properties in insect saliva by a mechanism not yet elucidated.

Supported by: CNPq, FAPERJ, INCT
Keywords:triatomine; microbiota-; hemostasis assays

PV052 - **STUDY OF THE BIOLOGICAL ALTERATIONS INDUCED BY A NOVEL SIRTUIN INHIBITOR ON LEISHMANIA AMAZONENSIS**

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Leishmaniasis is one of the most important neglected diseases, which is endemic in 98 countries worldwide and is caused by protozoan parasites of Leishmania genus. There are three major clinical manifestations: visceral, cutaneous and mucocutaneous. The chemotherapy includes the pentavalent antimonials, amphotericin B, pentamidine and miltefosine, however, the high levels of toxicity, long time of drug administration and cases of resistance makes necessary the development of new compounds. Recent works have demonstrated the potential of class III deacetylases histones (NAD+)-dependent inhibitors for the treatment of several types of cancer. Sirtuins are enzymes with significant regulatory role that involved ADP ribosylation and/or NAD+-dependent protein acetylation (including histones and non-histones proteins). Thus, sirtuins act as an important regulator of gene transcription with central role in many cytoplasmic events. The aim of this study was to study the effects of a novel sirtuin inhibitor, NIH119, against Leishmania amazonensis. Using different techniques, we evaluated the effects of NIH119 in the growth, morphology and ultrastructure. Promastigotes were significantly affected by the treatment, presenting IC50 around 1 µM; NIH199 was also active against intracellular amastigotes. Scanning electron microscopy and optical microscopy revealed changes in the cell body morphology of the promastigotes. Immunofluorescence microscopy indicated possible abnormal chromatin condensation, which was also observed by transmission electron microscopy. In addition, some images indicated alterations in the mitochondrion and presence of several small vesicles inside the flagellar pocket, indicating a possible increase in the secretory pathway. At this moment, cell viability and cell cycle analyses, as well other microscopic techniques are being performed to better understand the mechanisms of action of NIH119 in Leishmania sp. **Supported by:**PIBIC
Keywords: Leishmaniasis; sirtuins; chemotherapy

PV053 - **INVESTIGATING LIPID METABOLISM IN MOSQUITO *AEDES AEGYPTI* INFECTED WITH *STRIGOMONAS CULICIS***

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Strigomonas culicis is a monoxenic protozoan found in the midgut of several species of mosquitoes, mainly in *Aedes aegypti*. It presents a life cycle restricted to the epimastigote form: fusiform, anterior kinetoplast, presence of undulating membrane and free flagellum. *S. Culicis* colonizes the insect's midgut after seven days of infection. After thirty-two days, some protozoa cross the space between epithelial cells, reaching the hemocoel of the insect and infect other organs such as salivary glands and fat body. Lipids are extremely important to cell membranes formation, as energetic substrate, cell signaling, inflammatory process, steroid hormones precursor, among others. This parasite is not able to synthesize the lipids necessary for its survival and proliferation, require to uptake from its host. Therefore, the aim of this work was to study the lipid metabolism in infected *Aedes aegypti* mosquitoes. Thus, mosquitoes (n=20/group) were infected with 10^7 parasites. After seven days post infection, fat bodies were removed. Samples were homogenized and subjected to lipid extraction and the main lipid classes were separated by the high performance thin layer chromatography technique. The results showed an increase of esterified cholesterol (51.21% vs 15.76%), triacylglycerol (25.37% vs 14.14%) and a decrease in free cholesterol (5.17 % vs. 10.55%), monoacylglycerol (2.98% vs 21.03%) and total phospholipids (10.53% vs 33.56%) in infected mosquitoes versus the control group, respectively. Thus, it's possible that infected mosquitoes with *S. culicis* are able to modulate the composition of the main lipid classes in fat bodies. Probably this modulation is serving to supply the parasites with lipids essential for their survival.
Supported by:CAPES, CNPq e FAPERJ **Keywords:**Lipid metabolism ; s.culicis; aedes aegypti

PV054 - ***STRIGOMONAS CULICIS* ANTIOXIDANT SYSTEM DURING THE INTERACTION WITH *AEDES AEGYPTI***

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Strigomonas culicis is a monoxenic protozoa found in the midgut of several mosquitoes, presenting a life cycle restricted to epimastigotes. Among its peculiarities, there is the presence of an endosymbiotic bacterium, but its role is not completely clear, being related to the supply of heme and iron. These protozoa colonize the midgut of hematophagous insects, a reactive oxygen species (ROS) enriched environment, and the comprehension of protozoa antioxidant pool is crucial for the protozoa-insect interactions success. Here, we analyzed *S. culicis* oxidative metabolism, comparing three different strains: aposymbiotic (Apo), wild type (WT) and H_2O_2-resistant wild type (WTR). Our previous data showed that Apo was more susceptible to pro-oxidants and mitochondrial inhibitors, and WT and WTR presented higher resistance to oxidative stress. WTR also produces reduced ROS levels, showing lower lipid peroxidation and an increase in antioxidant activity. Furthermore, WTR also presented an increase in cytosolic and mitochondrial tryparedoxin peroxidase isoforms and tripanothione reductase transcripts levels, while symbiont elimination caused a decrease in gene expression of tripanothione synthase and tripanothione reductase without modulation of tryparedoxin peroxidase expression. WTR presented a greater colonization of *Aedes aegypti* midgut *in vivo*, reinforcing the hypothesis that the pro-oxidant environment in the mosquito gut, maintained through sucrose feed, regulates *S. culicis* population, data reinforced by the increase in the three strains gut colonization after *A. aegypti* feeding with ascorbate *ad libitum*. However, the blood fed led to decrease in the *A. aegypti* infection by the three strains up to 11 days post infection (dpi), which was reversed after 21 dpi, especially during WTR infection. **Supported by:**FAPERJ, CNPq and FIOCRUZ **Keywords:**Strigomonas culicis; antioxidant system; aedes aegypti

PV055 - **MOLECULAR CHARACTERIZATION OF IRON TRANSPORT IN *TRYPANOSOMA CRUZI* AND ITS POTENTIAL ROLE IN PARASITE VIRULENCE**

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Chagas' disease is caused by the parasite protozoan *Trypanosoma cruzi*. Historically, Chagas' disease was concentrated in some areas of Latin America where poor housing conditions favor vector infestation. Regarding the protozoan *T. cruzi*, it requires both heme and nonheme iron (Fe) sources for an optimal growth rate. Fe is a micronutrient that plays an essential role in almost all living organisms, being an essential cofactor in many metabolic pathways and enzymes. Fe is also harmful due to its capability in catalyzing the formation of reactive O_2 species (ROS) and, for this reason, all living systems have developed tight mechanisms for the control of uptake, metabolism and storage of Fe. However, there is very limited information available regarding Fe sources and Fe uptake by *T. cruzi*.
Intracellular Fe concentration is lower in cells maintained in Fe-depleted medium than in those that were grown in control conditions with micromolar Fe. This result is accompanied by a lower O_2 consumption by the *T. cruzi* cells that were maintained in Fe-depleted medium, thus revealing a decreased oxidative metabolic rate. We identified a putative Fe transporter in *T. cruzi* genome that we denominated TcIT. This transporter is homologous to the recently described Fe transporter in *Leishmania amazonensis*, LIT, and to the *Arabdopsis thaliana* Fe transporter IRT1. Epimastigotes grown in Fe-depleted medium presents increased TcIT transcript when compared to epimastigotes grown in control medium. TcIT is localized in the epimastigotes plasma membrane, as demonstrated by immunofluorescence microscopy within cells expressing TcIT-HA.
We conclude that the present data regarding the mechanisms of non-heme Fe transport at cellular and molecular levels in *T. cruzi* open new vistas on the field of Fe metabolism and on the role of ionic Fe transport in virulence and parasite proliferation.
Supported by:CNPq **Keywords:**Iron transport; trypanosoma cruzi; iron metabolism

PV056 - ***LEISHMANIA INFANTUM CHAGASI* ACTIVATES THE JAK-STAT IMMUNE PATHWAY IN *LUTZOMYIA LONGIPALPIS* LL5 EMBRYONIC CELLS AND REDUCES STAT EXPRESSION IN ADULT FEMALE FLIES**

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Visceral leishmaniasis is caused by *Leishmania infantum chagasi* and in Brazil transmitted mostly by *Lutzomyia longipalpis*. Insect immunity studies contribute to understand how these vectors control microbial infections. Toll, IMD and JAK-STAT pathways are involved in innate immunity in insects and regulate effector molecules such as antimicrobial peptides (AMPs). The JAK-STAT pathway is related to antiviral or to *Plasmodium* responses in *Drosophila* or *Anopheles aquasalis*, respectively. We are investigating this pathway role in the *L. longipalpis* response to *Leishmania* infection. We assessed the relative gene expression of the JAK-STAT negative regulators Pias, PTP and SOCS, the transcriptional factor STAT and effector genes attacin and VIR-1 in *L. longipalpis* embryonic cells LL5 and adult insects by qPCR. All target genes were overexpressed in LL5 cells 12h post challenge, indicating that JAK-STAT pathway was activated by the presence of Leishmania. Furthermore, in LL5 cells the silencing of PIAS caused increased expression of the transcriptional factor STAT and the effector genes Vir-1 and attacin while the negative regulators PTP and SOCS expression decreased indicating that silencing of PIAS could activate the pathway. In *Leishmania* infected females STAT expression decreased at 48 and 72h post infection. We also tested the gut microbiota counterpart. Our results showed that antibiotic feeding did not interfere with JAK-STAT activation, but the expression of attacin gradually decreased. Together our results show that the JAK-STAT pathway is activated in LL5 cells in response to *Leishmania* in early times and the silencing of PIAS can activate the pathway. In *Leishmania* infected flies the transcription factor STAT was repressed. We are currently investigating whether PIAS or other repressor molecules are involved in repressing STAT. **Supported by:**CAPES
Keywords: Lutzomyia longipalpis; leishmania; immunity

PV057 - **AUTOPHAGIC INDUCTION AND MITOCHONDRIAL REMODELING IN *TRYPANOSOMA CRUZI* EPIMASTIGOTES UNDER NUTRITIONAL AND PH STRESS**

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Chagas disease, caused by the protozoan *Trypanosoma cruzi*, is a neglected illness that affects millions of people in Latin America, being an emergent illness in non-endemic countries due to the triatomine-independent transmission routes and the immigration globalization. During the parasite life cycle, stress conditions such as pH and nutritional changes induce protozoan differentiation: epimastigotes to metacyclic trypomastigotes in insect, and trypomastigotes to amastigotes in mammals. Autophagy is a constitutive recycling process of cell components and could remodel parasite organelles such as mitochondrion during stress situations. In this work, we evaluated the role of mitochondrion, oxygen reactive species production and its relation with autophagy in epimastigotes submitted to nutritional stress and pH variation by different biochemical and molecular approaches. After 24 and 96h, only nutritional stress induces an important reduction in oxygen uptake. On the other hand, nutritional and pH stress decrease citrate synthase activity after 24h. qPCR data shows a remarkable increase in citrate synthase, complexes II, III and IV transcripts levels in parasites submitted to pH stress as well as trypanothione reductase and tryparredoxin peroxidase contents. Atg8 expression was also increased in epimastigotes under nutritional and pH stress (both parameters % Atg8+ parasites and number puncta/parasite). However, further experiments must be performed to confirm these data. The comprehension of the relation between autophagy and mitochondrial functionality in different stress conditions, especially in trypanosomatids as *T. cruzi* that present a single organelle could be crucial to the development of new anti-parasitic strategies.
Supported by:FAPERJ, CNPq and FIOCRUZ. **Keywords:**T. cruzi; mitochondrion ; autophagy

PV058 - **PRODUCTION OF TRYPANOSOMA CRUZI LINEAGES EXPRESSING A PANEL OF WILD-TYPE AND MUTANT VERSIONS OF THE TCRAB14 GTPASE**

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Rab family GTPases are fundamental to intracellular transport, regulating traffic events in both endocytic and exocytic pathways. Rab proteins undergo a GTPase activation cycle, in which they are active when bound to GTP and inactive after GTP hydrolysis. These proteins are also modified with geranylgeranyl tails on two C-terminal cysteine residues, which are crucial for membrane association and function. Rab14 was one of the last Rabs to have its functions investigated in animals and almost nothing is known about it in other models. In mammalian cells it associates with Golgi and early endosomes, being also involved in phagosome maturation. We initiate the characterization of Trypanosoma cruzi TcRab14 by producing parasite lineages expressing versions of GFP-tagged TcRab14. Besides the wild-type TcRab14 (WT), we have cloned TcRab14-Q65K (GTP-locked dominant positive), TcRab14-S17N (GDP-locked dominant negative), TcRab14-dCxC (prenylation defective) and TcRab14-Q65K-dCxC (GTP-locked/prenylation defective). These genes were cloned in the pTEX-GFPn vector to produce constructs fused with the GFP gene. These constructs were transfected in epimastigotes of Dm28c T. cruzi by electroporation. Epimastigote lineages expressing these constructs were selected with G418 and expression of recombinant genes was confirmed by RT-PCR. Fluorescence microscopy provided a first glimpse on Rab14 subcellular localization. The GFP-TcRab14-WT presented a punctate vesicular distribution in the anterior region, whereas the GFP-TcRab14-S20N mutant exhibited vesicular distribution spread through the cell. The GFP-TcRab14-Q65K mutant presented intense fluorescence in the kinetoplast surrounding region and prenylation deficient mutants exhibited disperse cytoplasmic distribution, with more intense signal close to the kinetoplast. More studies are necessary to access its precise cellular localization and dinamics, what will be crucial to determine the role of TcRab14 in T. cruzi physiology. **Supported by:**FUNEMAC - Fundação Educacional de Macaé (IC fellowship) **Keywords:**Trypanosoma cruzi; rab; gtpase

PV059 - **CHARACTERIZATION OF TGHDAC2 AND TGHDAC4, SPECIFIC OF APICOMPLEXA.**

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Epigenetic regulation is one of the key mechanisms of gene expression control and *Toxoplasma gondii* (*T. gondii*) has an extensive repertoire of enzymes for this pathway. The focus of our group are *Toxoplasma's* histones deacetylases (HDACs), since their function are related with gene silencing in other eukaryotes. *Toxoplasma* has seven HDCAs and we are particularly interested in TgHDAC2 and TgHDAC4, both specific of Apicomplexa parasites. TgHDAC2 has a long HDAC domain, interposed with a region of 200 amino acid long, unique of Apicomplexa. We obtained the gene knockout that was replaced by *hxgprt* gene through homologous recombination. So far, we observed a lower proliferation rate of Δ*tghdac-2* parasites, suggesting a role during cell cycle progression. In addition, Δ*tghdac-2* parasites showed a decrease in virulence, when compared with wild type parasites. We are currently investigating the differences in DNA replication in the knockout strain and obtaining antibodies to localize this protein in *Toxoplasma*. On the other hand, TgHDAC4 is a class IV HDAC, the less characterized class of deacetylases. To explore the function of TgHDAC4, we constructed an endogenous tag line. By immunofluorescence assay, we found that the protein colocalizes with the apicoplast, a derived plastid found in Apicomplexa. TgHDAC4 also colocalizes with TgHU, a histone-like protein, homolog to the bacterial HU. By Western blot, we observed a protein about 20 kDa smaller than expected, common in apicoplast proteins due to cleavage signal and transit peptides. We are now focusing on the TgHDAC4 partners identification by immunoprecipitation assay followed by mass spectrometry. In addition, we intend to produce antibodies against TgHDAC4 to confirm the localization and correct size of TgHDAC4 in *Toxoplasma*. Understanding how this protein works could provide new insights into the metabolism of the apicoplast, an organelle essential for parasite survival. **Supported by:**CAPES, CNPQ, Fundação Araucária, ICC/Fiocruz-PR **Keywords:**Toxoplasma; histone deacetylase; apicoplast

PV060 - **RESEARCH OF SPECIFIC AND ESSENTIAL COMPONENTS FOR EXPORT OF TRYPANOSOMATID MRNA**

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The nucleo-cytoplasmic RNA export is an essential pathway for gene expression regulation in eukaryotic cells but it is still poorly understood in protozoan parasites. Bioinformatic comparisons indicated that most of the known proteins are not conserved in several species of parasites. One exception is the mammalian protein UAP56 (Sub2, in yeast) which the orthologue in T.cruzi (TcSub2) is essential for parasite survival and export of mRNA. Proteomic approaches allowed the identification of factors associated with TcSub2 that seems to be exclusive of trypanosomatid species. These evidences support the presence of divergent components in the mRNA export route in trypanosomatids. Among them, two hypothetical proteins presented conserved domains and were nominated as TcFOP-like and TcAPI5-like. TcFOP-like contain a FOP domain, which is characterized by the presence of a UBM-Uap56/Sub2 Binding Motif, while TcAPI5-like contain an API5 domain which is present in Apoptosis Inhibitory 5 protein. Our main goal is to evaluate the function of these proteins as components of mRNA export route. We confirmed that TcFOP-like and TcAPI5-like are nuclear proteins and colocalize with TcSub2. We are currently performing immunoprecipitation assays in order to confirm the interaction of TcFOP-like and TcAPI5-like with TcSub2 and other mRNA export factors in addition to the identification of new components by proteomic analysis. Furthermore, genetic reverse tools will enable the functional analyse of non-conserved proteins for mRNA export. The essential and exclusive proteins will be submitted to crystallization trials in order to elucidate their structure and provide data regarding their mechanism of action.

Supported by:CAPES, CNPq, FIOCRUZ, Fundação Araucária **Keywords:**Tcsub2; tcfop-like; tcapi5-like

PV061 - DEVELOPMENT OF A MOLECULAR ASSAY FOR DIFFERENTIAL QUANTIFICATION OF PARASITE LOAD IN RHODNIUS PROLIXUS INFECTED WITH TRYPANOSOMA CRUZI

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Chagas disease constitutes the fourth most important tropical disease, supplanted only by malaria, tuberculosis, and schistosomiasis. Microscopical examination is a classic method to describe the natural infection of triatomines by Trypanosoma cruzi, but it presents some limitations, such as low sensitivity and reproducibility. In this context, the application of a quantitative Real Time PCR (qPCR) assay can improve sensitivity, specificity and reproducibility, in order to investigate the development of T. cruzi in vectors. In this work, a qPCR TaqMan multiplex assay targeting T. cruzi nuclear satellite DNA and triatomine 12S rRNA gene was developed, which is capable to detect and quantify absolute levels of T. cruzi in triatomines, but is unable to differentiate between live and dead parasites. It means that a positive result does not imply pathogen viability. Thus, in order to quantify only viable parasites and to distinguish trypomastigote forms and epismastigotes forms, a RT-qPCR was also developed, targeting T. cruzi GAPDH and TcS5 genes, highly expressed in trypomastigotes forms. To follow T. cruzi (Dm28c) development in Rhodnius prolixus total digestive tract and its different portions, total DNA and RNA were extracted from insect samples under increasing periods after feeding with blood containing T. cruzi. Nine days after feeding, it was possible to observe a time-dependent decreasing on the viable parasite quantity during the time course of the experiment, in which this decreasing occurs mainly in the anterior midgut portion. At the same time, was also detected 205,91 equivalents of trypomastigotes forms/triatomine inside the entire digestive tract of the insect vector four weeks after feeding. The qPCR and RT-qPCR assays are suitable to determine the infection rate of triatomines and to differentially quantify with high sensitivity and precision the parasite load, total and viable, in triatomines.
Keywords: Qpcr rt-qpcr; trypanosoma cruzi; rhodnius prolixus

PV062 - CRYSTAL VIOLET CHEMICAL ANALOGS INHIBIT THE *TRYPANOSOMA CRUZI* PROLINE TRANSPORTER AND EXERT TRYPANOCIDAL EFFECT.

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Trypanosoma cruzi, the etiological agent of Chagas disease, has a metabolism largely based on the consumption of glucose and proline. Additionally, this amino acid is involved in differentiation processes, cellular invasion and resistance to several stresses as oxidative, metabolic and osmotic. We have previously identified the proline permease TcAAAP069 in *T. cruzi* and we showed the effect of inhibition and/or blockage of proline transporter TcAAAP069 on parasite survival. Crystal violet (CV) was used for several years as a blood additive for prevention of transfusion transmitted Chagas disease and the protein synthesis inhibition observed by CV could be due to inhibition of amino acid uptake. In this work, we demonstrated that CV enters at least in part through the proline permease TcAAAP069. CV inhibited proline transport and parasites overexpressing proline transporter were more sensitive to its trypanocidal action. The protective effect of proline in oxidative stress situations led to a 3-fold increase resistance when proline was added to the medium along with CV. In order to obtain other drugs that might have similar effects to CV, we performed a similarity-based virtual screening, using the CV as query and different drug databases. So far, two of the obtained compounds proved to be effective as proline transport inhibitors that also had trypanocidal effect. Another drug obtained with this strategy inhibited TcAAAP069 activity but lacked trypanocidal effect. Taken together, these results show that it is possible to obtain new trypanocidal compounds by drug repositioning using CV as a reference. Drug repositioning is one of the recommended strategies by the World Health Organization to fight neglected diseases, like Chagas disease, because costs and development time are significantly reduced for its application in therapy. **Supported by:**CONICET **Keywords:**Trypanosoma cruzi; crystal violet; drug repositioning

PV063 - **STUDY OF PHOSPHOLIPASE A2 OF *RHODNIUS PROLIXUS* GUT DURING DIGESTION**

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Chagas disease is one of tropical neglected diseases, which affects approximately 6 to 8 million people worldwide. Such disease has as an etiologic agent a parasite named *Trypanosoma cruzi*, which has a life cycle involving a vertebrate and an invertebrate host. One of invertebrate host is *Rhodnius prolixus*, a haematophagous insect of Triatominae subfamily which is capable of feeding any warm-blooded vertebrate host. Fighting against the vector is still the best way to prevent this disease, and because of that, finding molecules with crucial role in the infection of the parasite is necessary. In this scenario, this study seeks to understand the role of phospholipase A_2 (PLA_2) from the gut of *Rhodnius prolixus* during blood digestion. We analyzed the activity of PLA_2 in epithelium and content of the anterior and posterior midgut, using enzymatic assays. It was possible to observe that in all studied samples and all days after feeding analyzed there was PLA_2 activity that belonged to different classes of this large family of enzymes. It has been seen that in the epithelium of the anterior and posterior midgut there was PLA_2 activity that possibly belonged to the class of secreted PLA_2 ($sPLA_2$). It was also observed, in the epithelium, PLA_2 activity of platelet activating factor - acetylhydrolases (PAF-AH) class, on the other hand, calcium independent PLA_2 ($iPLA_2$) activity was observed only in the epithelium of the anterior midgut. In the content was observed PLA_2 activity that possibly belongs to the $sPLA_2$ and PAF-AH classes. Therefore, this work contributes to the better understanding of the role of PLA_2 during digestion in insects and to understand their role in generate products that are important for establishment of infection and at the same time for insect defense mechanisms. **Supported by:**CNPq, FAPERJ, CAPES
Keywords: Rhodnius prolixus; phospholipase a2; lipids

PV064 - ***TRYPANOSOMA CRUZI* ACID PHOSPHATASE TYPE 5 (ACP5) BLOCKS MACROPHAGE OXIDATIVE METABOLITE PRODUCTION**

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Chaga's disease is a major health problem in Central and South Americas. Recently, cases in South America and in Europe have become a global disease problem. *Trypansoma cruzi* (the causative agent of Chaga's disease) presents a complex life cycle that involves adaptations in vertebrate and invertebrate host. As a protozoan parasite of hematophagous insects and mammalian hosts, *T. cruzi* is exposed to reactive species of oxygen. In silico analysis of the *T. cruzi* genome showed the presence of an acid phosphatase type 5 (TcACP5) with high homology to the human ACP5. To biochemical and the modulatory analyses we used a heterologous expression system to purify the enzyme. TcACP5 hydrolyzed p-nitrophenylphosphate (*p*NPP) at a rate of 41.2 ± 1.7 nmol pNPP × h^{-1} × (µg/ µL $PTN)^{-1}$. Values of $V_{máx}$ and apparent K_m for *p*NPP hydrolysis were 48.1 ± 1.6 nmol *p*NPP × h^{-1} × (µg/ µL $PTN)^{-1}$ and 360.5 ± 37.8 µM *p*NPP, respectively. The maximal phosphatase activity was obtained in acid pH (5.5). In addition, the phosphatase was strongly inhibited by inhibitors of acid phosphatases. Different phosphorylated amino acids were used as substrates and the highest rate of phosphate hydrolysis was achieved using phosphothreonine and phosphoserine. We also demonstrated that TcACP5 is able to metabolize hydrogen peroxide (H_2O_2) in in vitro experiment. Moreover, this enzyme decrease rate of H_2O_2 generation by macrophage. Taken together, suggest the essentiality of TcACP5 in the physiology of *T. cruzi*.
Supported by:CNPq
Keywords: Trypanosoma cruzi; acid phosphatase ; hydrogen peroxide

PV065 - **TCFR1 FERRIC IRON REDUCTASE OF *TRYPANOSOMA CRUZI* IS INVOLVED IN FE HOMEOSTASIS**

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Trypanosoma cruzi is a parasitic protozoan that infects a various species of domestic and wild animals, triatomine bugs and humans. This parasite is the etiologic agent of American tripanossomiasis, also known as Chagas´ Disease and it affects about 17 million people in Latin America. *T. cruzi* presents high requirement for iron (Fe), mobilizing heminic and nonheminic iron. Fe is a crucial micronutrient for almost all the existing cells, and can act as a cofactor for several enzymatic activities and metabolic pathways. Fe^{2+} ion, which is soluble in neutral pH, cannot be accumulated due to the generation of reactive oxygen species (ROS), through Fenton Reaction. For that reason, Fe is founded in a slightly soluble ionic form (Fe^{3+}) in aerobics environments. Furthermore, Fe^{2+} transporters act together with Fe-reductases and these latter are essential for the generation of soluble Fe^{2+} substrate translocated across the plasma membrane.
Here, we identified a putative Fe-reductase in the *T. cruzi* genome, TcFR1. This enzyme is homologue to LFR1, a Leishmania amazonensis Fe-reductase, presenting 40% of identity and 58 % of similarity, with 35.64 kDa. TcFR1 is crucial for Fe^{3+} to Fe^{2+} reduction, an obligatory redox transition that happens before incorporation of Fe by tripanosomatides. Fe^{3+} to Fe^{2+} reduction, catalyzed by TcFR1 *T. cruzi* epimastigotes, was measured using potassium ferricyanide ($K_3Fe(CN)_6$), a colorimetric substrate. TcFR1 activity and gene expression were evaluated in *T. cruzi* epimastigotes grown at different Fe concentration in culture media.
Thereby, this work will allow elucidating the Fe ion transport system in *T. cruzi*, which is coupled with the Fe-reductase, as well as the possible involvement of this system in parasite virulence.
Supported by:FAPERJ e CNPQ **Keywords:**Fe-reductase; trypanosoma cruzi; iron metabolism

PV066 - **FUNCTIONAL CHARACTERIZATION OF *RHODNIUS PROLIXUS* CHITINASE**

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Chagas disease is a parasitic disease caused by the protozoan Trypanosoma cruzi. Rhodnius prolixus is the main vector of T. cruzi in the northern region of South America. There are nine chitinase genes in R. prolixus genome. Insect chitinases belong to glycoside hydrolase family 18 (GHF18) and hydrolyse glycosidic linkages within chitin chains during relocation of chitin in development and molting, digestion in detritivorous and predators, and control of the thickness of the peritrophic membrane. Through phylogenetic analysis, we found that R. prolixus chitinases belong to different GHF18 subgroups previously described. Knowledge of chitinases roles may reveal new targets for vector control and new aspects of their physiology. Our goal is the detailed study of the expression of R. prolixus chitinases, as well as analysis of their physiological role through silencing by RNAi. We performed a semi-quantitative analysis of GH18 gene expression in all developmental stages, but significant differences were found only in the egg, where RpCht9 was more expressed. Comparing different tissues, RpCht8 was more expressed in salivary glands and hindgut, RpCht3/RpCht5 were less expressed in the fat body and posterior midgut, and RpCht3/RpCht7 were less expressed in the anterior midgut. The RpCht7 gene was chosen for functional analysis because it showed a higher rate of silencing after treatment with dsRNA. RpCht7 knock down doubled the mortality in starving fifth instar nymphs when compared to dsGFP-injected controls. However, it does not alter blood intake, diuresis, digestion, molting rate, molting defects, sexual ratio, percentage of hatching and mean hatching time. Nevertheless, RpCht7 silencing reduces female oviposition in 34%, and differences in oviposition occur within 14 to 20 days after a regurgitating blood meal. These results suggest that RpCht7 is involved in R. prolixus reproduction and might be a promising target for manipulation of vector fitness. **Supported by:**FIOCRUZ, FAPERJ, CNPq, and CAPES
Keywords: Rhodnius prolixus; chitinases; rnai

PV067 - **TRANSMISSION-BLOCKING ATTRACTIVE SUGAR BAITS: A NEW PERSPECTIVE FOR THE CONTROL OF LEISHMANIASIS**

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Lutzomyia longipalpis is the main vector of American Visceral Leishmaniasis, a disease caused by Leishmania parasites. Adult sand flies feed on blood or plant sugars, but their carbohydrate digestion is poorly characterized. Plants produce beta-glycosides as a strategy against herbivory, releasing toxic compounds after their hydrolysis in the insect gut. beta-glycosidase and trehalase are the molecular targets of beta-glycosides in other insects, and are important enzymes in sand fly sugar metabolism. In this respect, it is possible that these glycosides affect sand fly physiology and Leishmania spp development inside its vector. Sugar meals containing amygdalin, esculin or mandelonitrile (0.1% w/v) affected the average life time of L. longipalpis, with respective reductions of 13%, 13% and 33% in females, and 0, 12% and 59% in males. Adult L. longipalpis hydrolyze esculin and feeding with this compound cause changes in their trehalase and beta-glucosidase activities. Female trehalase activity is inhibited in vitro by esculin. Esculin is fluorescent, so its ingestion may be detected and quantified in whole insects or tissue samples. Amygdalin and esculin have no effect in vitro against L. amazonensis, L. braziliensis, L. infantum and L. mexicana, while mandelonitrile significantly reduces the viability of all species. Esculetin similarly reduces parasite numbers of L. infantum and L. mexicana. L. longipalpis females fed on mandelonitrile 0.1% (7.5 mM) had a reduction in the number of parasites/gut and prevalence of infection after 7 days of infection with L. mexicana (by counting and qPCR). Mandelonitrile showed no anti-feeding effect or repellent activity, and do not affect the ingested sugar amounts. Some plant glycosides have important effects on L. longipalpis physiology, affecting the development of parasites in culture and inside the vector. These findings may form the basis for development of new strategies for control the transmission of Leishmaniasis. **Keywords:**Lutzomyia longipalpis; beta-glycosides ; leishmania

PV068 - **INORGANIC PHOSPHATE UPTAKE IN *TRYPANSOMA CRUZI* IS MODULATED BY HYPOSMOTIC SHOCK**

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INTRODUTION: *Trypanosoma cruzi* is the etiologic agent of Chagas's disease, and its complex life cycle switches between reduviid insect vectors and mammals. During this complex life cycle, *T. cruzi* are usually subjected to hyposmotic environments. In order to adapt to these osmotic variations, *T. cruzi* present mechanisms that involve different signaling pathways. The contractile vacuole complex (CVC) collects and expels excess water as a mechanism of regulatory volume after hyposmotic stress, while acidocalcisome rapid hydrolysis polyP during hyposmotic stress. Moreover, under hyposmotic shock, the acidocalcisome fuse with CVC and inorganic phosphate (P_i) is released due to the cleavage of polyP present in acidocalcisomes. OBJECTIVE: The aim of this work is investigate the influence of hyposmotic shock on P_i uptake in *T. cruzi*. RESULTS: The inorganic phosphate uptake decreased approximately 65% while intracellular polyP content decreased 20% and intracellular P_i content increased 50%. Furthermore, important changes in cell volume were observed. CONCLUSION: These results suggest that hydrolysis of polyP during hyposmotic shock is related with intracellular P_i balance by regulation of P_i uptake in *T. cruzi*. **Supported by:**CAPES
Keywords: Trypanosoma cruzi; hyposmotic shock; inorganic phosphate uptake

PV069 - **INSIGHTS INTO THE EFFECTS OF THE TOLL PATHWAY REGULATION ON LUTZOMYIA LONGIPALPIS, THE MAIN VECTOR OF VISCERAL LEISHMANIASIS IN BRAZIL.**

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Visceral Leishmaniasis is caused by the protozoan parasites Leishmania donovani and Leishmania infantum chagasi and is a potentially fatal disease with a worldwide distribution. Transmission occurs through the bite of female phlebotomine sandflies and the principal vector in the Americas is Lutzomyia longipalpis.
Understanding parasite-vector interactions could provide new approaches for the development of alternative vector control or transmission blocking strategies. Our group showed recently that L. longipalpis LL5 embryonic cells have active Toll and IMD pathways that exhibited increased antimicrobial peptide (AMP) expression after their negative regulators (cactus and caspar) were silenced and also with Leishmania challenge. Artificial infection of female sand flies elicited an increase in AMPs and cactus expression suggesting possible participation of the pathway in infection response. The objective of this project is to evaluate the effects of the Toll pathway regulation in the vector during infection with L. i. chagasi.
Cactus silencing in female L. longipalpis did not elicit up-regulation of AMPs as observed in in-vitro model, suggesting that another negative regulator as Wnt-like could be involved. Single and double silencing of cactus and Wnt was carried out using RNAi and assessed by qPCR, showing significant reduction in gene expression up to three days post injection. Relative expression of cecropin, defensin-2 and attacin in all silenced groups was assessed, all three AMPs presented up-regulation in the first 48 hours post injection only in double silenced flies. Additionally, co-silenced female sand flies were infected with L. chagasi and parasite load was assessed by qPCR using relative expression of Leishmania actin. There was significant infection reduction at 24 hours, consistent with the increase of AMPs observed in previous experiment. Our results indicate that activated Toll pathway can reduce Leishmania survival in the vector. **Keywords:**Toll ; l.longipalpis; l.i.chagasi

PV070 - **COMPARATIVE ANALYSIS OF THE STEROL BIOSYNTHESIS PATHWAY IN TRYPANOSOMATID PARASITES OF NON-HUMAN HOSTS**

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Sterols play important roles in the organization, dynamic and functionality of cell membranes. *T. cruzi* and *Leishmania* spp. have the machinery to produce ergosterol-derived sterols, which differs from the cholesterol by the presence of a methyl group at C24. In other trypanosomatids, the sterol biosynthetic pathway remains not fully known, although it is presumed that they are able to produce ergosterol. In this way, the aim of this work is to elucidate and describe the sterol biosynthetic pathway in *Crithidia fasciculata*, *Leptomonas pyrrhocoris* and *Phytomonas serpens*, that present different hosts. Thereunto, two strategies were used: the search of orthologs in KEGG pathway database using BLAST and the use of Gas Chromatography-Mass Spectrometry (GC-MS) to evaluate sterol profile in cholesterol-rich (with fetal bovine serum – FBS) and in cholesterol-free (without FBS or with delipidated FBS for one passage) conditions. As results, we observed that, among the three studied parasites, *C. fasciculata* is the most adapted trypanosomatid for surviving in the presence and absence of exogenous sterols. We found 13 orthologous genes of sterol pathway in *C. fasciculata* and detected the presence of ergosterol and other ergostane-derived sterols by GC-MS. We also noted the presence of cholesterol in different culture conditions, suggesting that this parasite can obtain this sterol from the medium. Similarly, *L. pyrrhocoris* presented the same sterol profile, but it demonstrated to be less resistant to absence of FBS, despite presenting more copy of the genes in its genome. *P. serpens* showed the slowest growth among these species. Moreover, the latter presented less diversity and number of copies of the studied genes, besides presenting a sterol profile slightly different, with presence of cholesterol even if when cultured in medium with delipidated FBS or without FBS, suggesting that this parasite should be able to store this sterol as a reserve. **Supported by:**CAPES, CNPq, FAPERJ
Keywords: Sterol biosynthesis; trypanosomatid; orthologous genes

PV071 - **EXPLORING THE ROLE OF HISTONE H2BV IN *TRYPANOSOMA CRUZI***

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Trypanosoma cruzi alternates between replicative and non-infective forms (epimastigotes and amastigotes) and non-replicative and infective forms (cellular and blood trypomastigotes). Differentiation between these forms is accompanied by differences in overall transcript levels and structural changes in chromatin. Several studies indicate that trypanosome chromatin contains several epigenetic alterations, including deposition of histone variants, enriched in putative transcription start sites. We recently observed that histone H2Bv of *T.cruzi* is enriched at chromatin from non-replicative forms, suggesting that it may play an important role in the phenotypic differences (gene expression and chromatin structure) observed between life forms of *T. cruzi*. Thus, the main goal of this work is to study the role of histone H2Bv in this parasite. To this end, we are performing pulldown assays to identify H2Bv interaction partners. The H2Bv recombinant protein was first purified by affinity nickel column and incubated with either epimastigotes or trypomastigotes extracts. The eluted proteins were fractionated by SDS-PAGE, trypsin-digested and submitted to LC-MS/MS. Several proteins were identified by MS analysis including a 15 kDa-protein, most probably a histone H2Az. To have a proper control for pulldown assays, we cloned the canonical H2B into pET-28a (+) for recombinant protein production. We have also generated H2Bv heterozigotous knockout parasites by homologous recombination to identify the importance and function of this gene. Homozygous mutant parasites were in inviable suggesting that H2Bv is an essential gene. We are currently evaluating the effects on proliferation and cell cycle of H2Bv heterozigotous knockouts parasites. **Supported by:**FAPESP / CAPES **Keywords:**Histone; proteomic; knockout

PV072 - **PROTEOMIC ANALYSIS OF EXOSOMES SECRETED BY LOGARITHMIC AND STATIONARY GROWTH PHASES OF LEISHMANIA INFANTUM CHAGASI.**

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Leishmania exosomes have been implicated in establishing infection in the vertebrate host by introducing proteins that target and down-regulate anti-parasitic immune pathways. However little is known regarding their interaction with the sand fly host. Recent studies have confirmed the in vivo release of Leishmania exosomes within the sand fly midgut and egestion during insect feeding. As such there is a compelling basis for research into the nature of exosome function within the sand fly vector.

In this project exosomes of Leishmania infantum chagasi – the principal agent of visceral leishmaniasis in the Americas - were isolated from logarithmic (LOG) and stationary (STAT) growth phases of cultured parasites and subjected to mass spectrometric analysis. A total of 1039 discrete peptide species were identified across both exosomes groups 615 and 354 peptides were identified in all LOG and STAT biological replicates respectively. 85 and 17 peptide species were found uniquely in LOG and STAT exosomes respectively. 86 and 13 peptide species were found in significantly higher abundance in LOG and STAT exosomes respectively. 30 peptides associated with parasite virulence, resistance and survival were present in greater abundance in LOG phase exosomes; among them GP63, Oligopeptidase B, EF1a, Calpain-like cysteine peptidase, APRT and Serine/threonine protein phosphatase. STAT exosomes exhibited few up-regulated peptides, none associated with parasite virulence, suggesting that any pro-parasitic functions are confined to the initial stages of sand fly infection. Peptide species were also subjected to ontological analysis in order to elucidate and ascribe clear functional roles to exosomes. Most notably, the proteins involved in several pathways employed by parasites to cope with energy, purine and oxidative stress were well represented among exosomes, suggesting that their functional role may be more extensive and complex than previously understood. **Keywords:**Exosomes; l. i. chagasi; proteomics

PV073 - **L. INFANTUM CHAGASI EXOSOMES AS IMMUNE-MODULATORS OF ENDOGENOUS L. LONGIPALPIS SHP-1 NEGATIVE TOLL REGULATOR.**

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Leishmania GP63 exerts a positive induction effect on the activation of vertebrate SHP-1 – a negative regulator of the Toll pathway – during infection. Recently our group confirmed the existence of an SHP-1 homologue in L. longipalpis, the vector of the visceral leishmaniasis agent, Leishmania infantum chagasi and that infection in this species exerts a positive effect on SHP-1 regulation. Additionally we have established the existence of GP63 in Leishmania exosomes suggesting that they may represent a principal source of parasite-driven immune-modulation in the sand fly during infection.
In this project exosomes of L. i. chagasi were isolated from logarithmic (LOG) and stationary (STAT) growth phases of cultured parasites and artificially fed at varying concentrations to female L. longipalpis in order to establish their effect on the regulation of host SHP-1 transcripts by RTqPCR.
Results showed that LOG exosomes exert a positive modulatory effect on L. longipalpis SHP-1 at low concentrations while higher concentrations appeared to exert little to no effect. STAT exosomes did not produce an increase in L. longipalpis SHP-1, which may be due to their lower relative abundance of GP63.
To this end, additional strategies are also being undertaken in order to elucidate the role of exosomes as immune modulators in the sand fly. RNAi-induced silencing of sand fly SHP1 is currently being investigated to determine whether its suppression has an effect on both exosome-induced immune modulation and the infection profile of the parasite itself. A recombinant GP63 protein is also being developed in order to discern the discrete role it may play in the sand fly midgut during infection. **Keywords:**L. longipalpis; exosomes; l. i. chagasi

PV074 - **IMMUNE RESPONSE TO ENTOMOPATHOGENIC FUNGUS *METARHIZIUM ANISOPLIAE* INFECTION IN *RHODNIUS PROLIXUS* (HEMIPTERA: REDUVIIDAE)**

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The *Rhodnius prolixus* is a hematophagous insect and vector of *Trypanosoma cruzi*, a protozoan parasite that causes Chagas disease. Insects are exposed to a wide range of microorganisms and have interconnected powerful immune reactions. Innate immunity is the first line of defense being divided into humoral response that is related to antimicrobial peptides (AMP), lectins and the prophenoloxidase cascade and the cellular response which includes phagocytosis, hemocytes aggregation and encapsulation of pathogens. The *Metarhizium anisopliae* is an entomopathogenic fungus used as biological control agents and start the infection process mainly by penetration through the insect cuticle. Here we have investigated the effect of *M. anisopliae* infection on the modulation immune response and to linking with embryogenesis process in *Rhodnius*. For this adults females of *R. prolixus*, in starvation or rabbit blood feeding, were challenged by conidial suspensions of *M. anisopliae* (1×10^7 conidia/mL) using a Potter tower and in both conditions after 24 and 72h, midguts and fat body were dissected. The modulation of immune responses was studied by analysis of the abundance of mRNAs encoding Dorsal and Cactus (Toll pathway), Relish (IMD pathway), Eigher (TNF ortholog), STAT and SOCS (Jack-STAT pathway) and Defensin and Lysozymes (LysA, LysB) (AMPs) by qPCR. The Toll pathway was activated in fat body of the blood meal insects 72h after infection. However, the transcripts level of LysA and LysB were up-regulated in midgut of fasting insects. Up-regulation of the Relish was also observed in the midgut blood-feeding, 72h after infection. We did not observe Eiger and STAT/SOCS expression significant at any time. Furthermore, we demonstrate that fungus infection did not interfere with insect survival, but DAPI staining analysis exhibited specific morphological defects. In this current study, we have implicated that the Toll and IMD pathways are involved in immunity against *M. anisopliae*. **Supported by:**CNPq / INCT-EM **Keywords:**Rhodnius prolixus; immune response; metarhizium anisopliae

PV075 - HEME-INDUCED ANTI-LEISHMANIAL ACTIVITY IN THE SAND FLY MODEL LUTZOMYIA LONGIPALPIS

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A successful Leishmania colonization of the sand fly midgut is dependent on several aspects such as physiological, physical and microbiological factors. The protozoa Leishmania needs to overcome initially the action of digestive enzymes. In addition, the parasites need to cope with reactive oxygen species (ROS) in the midgut. The peritrophic matrix (PM) is a semi-permeable structure that encases the food bolus. This PM plays important roles such as compartmentalizes the digestive process and also protects the gut wall from the direct contact with food-derived molecules and potential pathogens. The aim of this work is to investigate the effect of heme on ROS production and Leishmania mexicana survival in the midgut of L. longipalpis. We disrupted PM formation feeding sand flies sheep blood supplemented with exogenous chitinase (1µg/mL) and Amplex red Kit was used to quantify hydrogen peroxide. Sand flies were also fed either on blood or plasma, with and without heme, seeded with 2 x 106 amastigotes/mL. Parasites were counted 6 days post-infection in a hemocytometer. The sand flies were also fed on sheep blood or phosphate buffer agarose solution with or without heme through chicken skin using an artificial feeder. The number of total L. mexicana was reduced in the midgut of chitinase treated sand flies. However, when sand flies were fed ad libitum from emergence on 70% sucrose supplemented with uric acid prior infection, the total number of parasites was rescued to levels similar to control. Heme led to an increase of hydrogen peroxide concentration in the midgut. When flies were fed on plasma seeded with amastigotes L. mexicana, the addition of heme caused a reduction of the total number of parasites per midgut. In conclusion, heme can induce ROS production when in contact with the gut wall, what is dangerous to the parasites and microbiota. The PM protects parasites and microbiota against the attack of ROS during the initial stage of blood digestion. **Supported by:**CNPq, INCT-EM
Keywords: Lutzomyia longipalpis; leishmania mexicana; heme

PV076 - THE ROLE OF THE ANTIMICROBIAL PEPTIDES OF *LUTZOMYIA LONGIPALPIS* IN THE CONTROL OF *LEISHMANIA INFANTUM* INFECTION.

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Visceral leishmaniasis is a chronic parasitic disease. In the Americas the etiological agent is *Leishmania infantum chagasi* and in Brazil the main vector is *Lutzomyia longipalpis*. Transmission occurs mainly through the bite of infected female sand flies. During development in the insect gut, the parasite encounters barriers such as digestive enzymatic activity, oxidative stress derived from blood digestion and immune responses of the insect. There are three pathways of innate immunity in insects: Toll, IMD and JAK-STAT. These pathways when activated trigger signaling cascades that culminate in the expression of antimicrobial peptides (AMPs) such as defensin, attacin and cecropin. Previously our group identified a *L. longipalpis* defensin with decreased expression at 7 days post infection when compared to its blood control group suggesting that the parasite may benefit from the reduced expression of this AMP at late infection phase. The objective of this project is to study the effect of silencing *L. longipalpis* AMPs on the establishment of *L. i. chagasi*. RNAi was used to silence attacin and defensing genes, previous to artificial infection with *Leishmania.* cDNA generated from silenced and infected insects was used to evaluate relative expression of these genes by quantitative PCR. Our preliminary results show that when attacin was silenced, a decrease in parasite load and an increase in bacteria were observed at 48h post infection. Regarding the silencing of defensin 2 there was increased survival of the parasite in the insect at 72h post infection. These data suggest that *L. longipalpis* attacin gene can be involved in the control of bacterial infection. More interestingly defensin 2 can be involved in the control of *Leishmania* infection.
Supported by:PIBIC **Keywords:**Leishmania; lutzomyia longipalpis; immunity

PV077 - IDENTIFICATION OF NOVEL MOTIFS REQUIRED FOR THE FUNCTION OF THE ESSENTIAL CAP BINDING PROTEIN HOMOLOG EIF4E3 IN LEISHMANIA SPECIES.

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Initiation of translation in trypanosomatids is a critical stage for post-transcriptional control, where different translation initiation factors (eIFs) are active. At this stage, a major step is the recognition of mature mRNAs by the proteins synthesis apparatus in order to translation to proceed. In eukaryotes, this event requires the cap binding protein, eIF4E, as part of the heterotrimeric eIF4F complex. In trypanosomatids, six eIF4E homologues have been described. Two of these, EIF4E3 and EIF4E4, are known to participate in the formation of eIF4F complexes involved in translation initiation and have been shown to be targeted by phosphorylation. The present work aimed to contribute to the understanding of EIF4E3 function, investigating its importance for cell viability of different Leishmania species and the role of selected motifs. Gene constructs were generated to allow knockout of the EIF4E3 gene copies in L. amazonensis and L. infantum. EIF4E3 was shown to be essential in both species. HA-tagged EIF4E3 wild type and mutants were also expressed in transgenic parasites in order to carry out complementation experiments in both Leishmania species, when double knockout of the endogenous gene is performed in the presence of the ectopic HA-tagged protein. Specific residues were identified as essential for EIF4E3 function and are critical for the survival of the organism. These include residues required for the protein to bind to the mRNA and to its partner EIF4G4 as well as a phosphorylation site found only in L. amazonensis EIF4E3. Other motifs, involved in the interaction with homologs of the poly-A binding protein (PABP) were also essential, while a putative phosphorylation site found in both Leishmania species did not interfere with EIF4E3 function. This study highlights the importance of investigating the role of eIF4E homologues in protein synthesis, as well as their role in the trypanosomatids cell biology in general. **Supported by:**FACEPE **Keywords:**Eif4e; trypanosomatids; regulation of gene expression

PV078 - EVOLUTIONARY RELATIONSHIPS FOR THE SUBUNITS OF THE TRANSLATION INITIATION COMPLEX EIF4F AND THE POLY-A BINDING PROTEIN (PABP) IN TRYPANOSOMATIDS

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During the initiation of protein synthesis in eukaryotes, the complex eIF4F and its partner PABP act as critical participants in selection of mRNAs to the ribosome for translation. The eIF4F complex is composed by the association of three subunits: eIF4E, the mRNA cap binding protein; eIF4A, an mRNA helicase; and eIF4G, a scaffolding protein. Interestingly, six eIF4E, five eIF4G and three PABP homologues have been described in Leishmania and Trypanosoma species, forming several eIF4F complexes with possibly different functions, indicating a high complexity for the control of translation initiation. Here we have taken advantage of the increasing availability of genome sequence data from different trypanosomatids and related organisms to investigate the evolutionary history of these complexes. First, the conserved multitude of eIF4Es reported for several is also present in related excavates, although the intrinsic characteristics of the six trypanosomatid homologues seem to be found only within kinetoplastids. For instance, the N-terminal extensions found in EIF4E3 and EIF4E4 are a unique characteristic within metakinetoplastids. Sequence comparison using the eIF4G data suggests that conserved features present within the five eIF4Gs from trypanosomatids are also exclusive to kinetoplastids. A low conservation in motifs involved in their interaction with eIF4E may be responsible for the selective binding between different eIF4E/eIF4G subunits and imply differences in the formation of the several eIF4F complexes. Additionally, the conservation of the three PABPs can be found within the Euglenozoan clade and denotes a very ancient gene duplication, which seems to be subsequently changed by the loss of the third PABP gene in some Trypanosoma species. These findings have answered relevant questions about the evolutionary history of the eIF4F-like complexes in trypanosomatids, while raising new ones regarding the diversity of the protein synthesis apparatus in eukaryotes.
Supported by:CNPq **Keywords:**Eif4f complex; translation initiation; trypanosomatids

PV079 - **MOLECULAR STRUCTURAL AND FUNCTIONAL ANALYSIS OF A PLATELET ACTIVATING FACTOR ACETYHYDROLASE (PAF-AH) OF *TRYPANOSOMA CRUZI***

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Chagas disease affects approximately eight million people. In the host vertebrate, the parasite is able to invade many cells, finding a favorable environment to continue its life cycle. Previous reports have linked phospholipase A_2 (PLA_2) activity to cell invasion events by several parasitical protozoa. PLA_2 enzymes constitute a superperfamily of proteins that contains various subgroups, including platelet activating factor acetylhydrolases. Some papers suggest the presence of a PLA_2 activity in culture of epimastigotes of *T. cruzi*. The goal of the present work was to investigate the presence of PLA_2 in *T. cruzi*, by Western blot (WB) and immunofluorescence, to measure the enzyme activity, as well as to construct a three-dimensional model of this protein. The WB and immunofluorescence were performed using epimastigotes of *T. cruzi* (Dm28c) and rabbit polyclonal IgG anti PAF-AH. In order to determine the PLA_2 activity, we also used extracts of epimastigotes and a kit. An in silico characterization of the protein encoded by the *T. cruzi* PLA_2 gene showed that this PLA2 is a PAF-AH. A band of 43 kDa, compatible with the molecular weight of PAF-AH, was shown by WB. The immunofluorescence showed the PAF-AH localized to the cytoplasm, surrounding the nucleus, as well as a faint label in the entire cell body, suggesting a higher concentration of PAF-AH in the endoplasmic reticulum. The PAF-AH assay showed that the cell lysate presents an activity of 0.1 mmol/min/ml and the specific PAF-AH inhibitor, abolished the activity. The model was constructed based upon the amino acid sequence submitted to the Phyre2 server. The validation was done using PROCHECK, which analyzes their stereochemical parameters. In summary, this is the first study to shed light to the molecular structure and function of a PAF-AH in *T. cruzi*. Ongoing investigations in our laboratory aim to lead the way for the use of this *T. cruzi* PAF-AH as a target for new chemotherapeutics against Chagas disease.

Supported by:CNPq, CAPES, FAPERJ and INCT-EM
Keywords: Modelling; lipase; phospholipids

PV080 - **DEVELOPMENTALLY REGULATED L-GLUTAMINE UPTAKE IN *TRYPANOSOMA CRUZI***

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T. cruzi is the causative agent of Chagas' disease and affects approximately 8 million people in the Americas. Amino acids are relevant to many biological processes beyond its obvious participation in protein biosynthesis. Remarkably, they are relevant for keeping the viability of different stages of *T. cruzi*, which undergo stress conditions along its life cycle. *T. cruzi* may obtain amino acids from biosynthesis pathways, or from their uptake from the extracellular medium. In this work, we biochemically characterized the Gln transport activity. It follows a Michaelis Menten kinetics, with a K_M of 1.29 ±0.08 mM and a V_{max} of 0.36 ± 0.05 nmols Gln/min.2 x 10^7 cells. The optimal pH for the transport activity was between 6.0 and 7.0 and it showed a nearly linear response to temperature in a range between 28 and 45 °C, which allowed us to calculate the activation energy for Gln uptake, being 63.29 ± 1.18 kJ/mol. In addition, we could demonstrate that Gln uptake is highly specific and active, with a specific activity depending on the nutritional state of the cell. In fact, the transport was dependent on the intracellular ATP levels and the H^+ gradient at the cytoplasmic membrane level. Finally, our results show that epimastigotes present the highest specific activity when compared to the others stages, followed by the intracellular epimastigote form. Interestingly, in those stages in which the Gln transport activity was maximal, the Gln synthetase activity was minimal, allowing us to infer a compensatory system. This data suggest that Gln uptake is an essential process at stages in which Gln biosynthesis is down-regulated.

Keywords: Trypanosoma cruzi; l-glutamine uptake; amino acid

PV081 - **RHABDOVIRUS INVESTIGATION IN SANDFLIES.**

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A considerable fraction of eukaryotic genomes is established by insertions of exogenous genetic elements. These retroelements are involved in evolutive adaptive genetic innovations. Some of these insertions can be environmentally favorable conferring advantages to the carrier. Here we identified rhabdoviral sequences integrated into the genome of LL5 cells, an embrionary cell lineage of *Lutzomyia longipalpis*, the main vector of visceral leishmaniasis (VL) in Brazil, and several other Brazilian sand flies species. Rhabdoviruses are enveloped, nonsegmented, single negative-stranded RNA belonging to the Rhabdoviridae family. They are largely distributed in nature infecting vertebrates, invertebrates and many species of plants. We identified two viral mRNAs transcribed from the Inserted Rhabdoviral Elements (IRE) coding for a nucleocapsid and a polymerase RNA dependent in LL5 cells. We investigated the presence of these IREs, and of their transcripts, in different phlebotomine populations from Brazil. We found that LL5 cells and natural wild phlebotomine populations share and transcribe the IRE coding for the nucleocapsid protein (NucIRE). Employing genomic PCR, RT-PCR and inverse PCR techniques followed by bioinformatic analyses we are determining the extension of these insertions in LL5 cells and natural wild Brazilians phlebotomine populations genomes. The majority of sand flies populations investigated presented the NucIRE except insects from Santarem/PA, Baturité/CE, Vicência, Paudalho, São Vicente Férrer, and Passira/PE, Lacerdopolis and UNOESC/SC. We are identifying flanking regions to know the viral fragment size. The polymerase fragment initially had 1,647bp and now is 2,554bp obtained by inverse PCR (the polymerase gene is about 6kb). The presence of these viral fragments in some but not all Brazilian sandflies raises important evolutionary questions and might be used as a tool for population genetic studies. **Supported by:**FAPERJ
Keywords: Sandflies; ll5 cells; rhabdovirus

PV082 - ***LEISHMANIA INFANTUM CHAGASI* JOURNEY INSIDE *LUTZOMYIA LONGIPALPIS* ASSESSED THROUGH GENE EXPRESSION**

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Leishmania infantum chagasi is the causative agent of visceral leishmaniasis and *Lutzomyia longipalpis* is the main vector in Brazil. The parasite is acquired when the female sand fly feeds on an infected host. The ingested parasites must resist blood digestion and differentiate into promastigote forms in the insect gut. Intense proteolytic activity occurs in the early hours of blood digestion, as well as oxidative stress caused by production of heme derived from hemoglobin digestion. After blood digestion parasites migrate to the anterior part of the insect gut and differentiate into metacyclic promastigote forms. Inside the gut, parasites must survive the immune responses and the interaction with the microbiota. We are investigating which *L. i. chagasi* molecules may play a role in the parasite-vector interaction. We performed qPCR to study *Leishmania* gene expression during promastigote initiated infection in *L. longipalpis*. At 1h and 72h post-infection the parasites abundantly expressed genes involved with differentiation and proliferation of the parasite such as glucose transporters and AAP3. Late times of infection showed increased virulence genes expression such as SHERP and GP63, or surface receptor such as LDL-receptor; decreased expression of XPRT that has a role in parasite multiplication. Furthermore, we investigated the role of the insect microbiota in parasite gene expression. Our results obtained from infected insects treated with antibiotics showed reduced bacteria loads in the gut, decreased expression of genes involved with metabolism of sugars and amino acids in early times and increased in late times during the infection. Our findings reflect aspects of parasite adaptation to the gut microenvironment and further transmission to the vertebrate host. Moreover the antibiotics treatment changed the gut microenvironment causing a rearrangement of *Leishmania* gene expression. **Supported by:**CAPES, IOC-Fiocruz
Keywords: Leishmania infantum chagasi; lutzomyia longipalpis; interaction

PV083 - **ROLE OF NF-κB TRANSCRIPTION FACTORS IN *RHODNIUS PROLIXUS* IMMUNE SYSTEM ACTIVATION IN RESPONSE TO BACTERIAL AND *TRYPANOSOMA CRUZI* INFECTION.**

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â€‹*Rhodnius prolixus* is a insect vector of *Trypanosoma cruzi*, the causative agent of Chagas disease in Latin America. Nuclear factor-κB (NF-κB) transcription factors (TF) are conserved components of innate immune system in several multicellular organisms including insects. The drug IMD-0354 (N-(3,5-bis-trifluoromethyl-phenyl)-5-chloro-2-hydroxy-benzamide) is a highly specific low molecular inhibitor of IkB kinases. It blocks IκBα phosphorylation, preventing nuclear translocation of the NF-κB TF. In insects, the immune system is activated upon microbial challenge, controlled by the immune deficiency (IMD), Toll and other immune signaling pathways, to combat infection. These activated pathways signal to downstream NF-κB TF to stimulate specific immune genes, triggering the synthesis of several molecules such as antimicrobial peptides (AMPs). The present work investigated the effects of oral administration of the drug IMD-0354 in *R. prolixus* immune responses challenged with different bacteria and *T. cruzi*. It was analyzed the insect mortality, the induction of AMPs, the antimicrobial activity in insect midgut samples using turbidimetric assays, as well as quantifying the relative expression of *R. prolixus* immune genes through RT-qPCR. Microbiota were quantified using colony forming unit (CFU) assays and viable parasites were counted in midgut insect samples using a Neubauer chamber under a light microscope. The treatment with IMD-0354 didn't altered the insect mortality rate. On the other hand, when the insects were treated with IMD-0354 and challenged with bacteria or *T. cruzi* throught blood meal, the insect mortality was increased while the number of infected insects was reduced. The drug also modulated the expression of several genes involved in *R. prolixus* immune signaling pathways. Additionally, it was observed an increase of cultivable bacterial microbiota in treated insects, highlighting the importance of NF-κB TF in *R. prolixus* intestinal homeostasis. **Supported by:**FAPERJ/CNPQ
Keywords: Rhodnius prolixus; immune system; trypanosoma cruzi

PV084 - **PRELIMINARY STUDIES ON THE EIF4B AND EIF5 TRANSLATION INITIATION FACTORS IN *LEISHMANIA INFANTUM***

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In eukaryotes, initiation of protein synthesis involves proteins known as initiation factors. Among these, we highlight the eIF4F complex (eIF4A, eIF4E, eIF4G) that acts facilitating the recognition of the mRNAs by the ribosomes to start translation. However, for this to occur other factors are required, such as eIF4B, which reinforces the helicase activity of eIF4A and eIF5, which acts during the recognition of the AUG start codon. Translation initiation in trypanosomatids is characterized by a multitude of homologues for the eIF4E and eIF4G subunits of eIF4F, indicating a diversity in function which might be associated with differential binding to putative partners, such as eIF4B and eIF5. This study therefore aimed to obtain the necessary tools for the *in vivo* characterization of eIF5 and of a possible eIF4B homologue in *Leishmania* species. Both proteins were overexpressed in *L. infantum* fused to an HA epitope, with their expression confirmed through western-blot assays of cellular extracts using an anti-HA antibody. For the putative eIF4B, the expressed protein revealed multiple isoforms, indicative of phosphorylation, as observed for the mammalian protein. The expression profile of these proteins was then evaluated during the life cycle of the parasite and no differences in abundance were observed for either of them. For eIF4B, however, the phosphorylated isoforms were mainly seen during the stationary phase of the growth culture. Immunoprecipitation assays were also performed using polyclonal antibodies to investigate the *in vivo* association of EIF5 and the possible eIF4B homologue with likely protein partners acting during translation. A suggestion of interaction was seen between eIF4B and a poly-A binding protein homologue (PABP1), but not with EIF4AI nor EIF5. These results should improve the understanding of how these factors act during protein synthesis in the trypanosomatids, aggregating relevant information on the translation initiation process.
Keywords: Trypanosomatids; eif4b; phosphorylation

PV085 - **IDENTIFICATION AND CHARACTERIZATION OF THE PROMOTER REGION OF LLCHIT1, A BLOOD-INDUCED MIDGUT CHITINASE GENE OF THE MAIN AMERICAN VISCERAL LEISHMANIASIS VECTOR, LUTZOMYIA LONGIPALPIS**

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American visceral leishmaniasis is a lethal disease caused by Leishmania infantum and transmitted by the sandfly Lutzomyia longipalpis. Ingestion of blood by the insect triggers the synthesis of peritrophic matrix (PM), which envelops the blood meal, aiding digestion. The PM represents a physical barrier the parasite must overcome in order to attach to the midgut epithelium and establish infection. In previous work our group identified a Lu. Longipalpis putatitve midgut-specific chitinase coding cDNA, named Llchit1, exhibiting peak expression 72h after blood feeding, coinciding with the degradation of the PM. A genomic library clone containing the Llchit1 sequence revealed a presumed regulatory region upstream of the start codon. In this work, different fragments of this region were submitted to a luciferase assay in vitro to assess their transcription regulatory activity. The -420 to +86 region generated 15 times more light than the vector without an insert. Deleting the first 180bp of the 5' portion of this fragment stopped its promoter activity without disrupting its main core promoter elements. This interval has 3 putative binding sites for GATA, a downstream product of the TOR pathway that activates the vitellogenin gene in mosquitos after a blood meal. Furthermore, the -881 to -871 region is a hypothetical binding site for the 20-hydroxyecdysone (20-HE) induced E74a transcription factor. In mosquitoes, there is an increase in 20-HE after blood ingestion. Exposure to 20-HE in vitro exacerbates the promoter activity of the fragment -1205 to +86 more than -420 to +86, which does not have the E74a binding site. Together, these results indicate that the expression of Llchit1 and the PM synthesis might be regulated by 20-HE and that perhaps the TOR pathway may also play a role in its activity. As Llchit1 seems to be a tissue-specific, blood-induced gene, its promoter represents a potential tool for the creation of expression vectors and transgenic sandflies. **Supported by:**CAPES
Keywords: Lutzomyia longipalpis; chitnase; promoter

PV086 - **DETECTION OF MULTIPLE CIRCULATING *LEISHMANIA* SPECIES IN *LUTZOMYIA LONGIPALPIS* IN THE CITY OF GOVERNADOR VALADARES, SOUTHEASTERN BRAZIL**

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Leishmaniasis encompasses a group of diverse clinical diseases caused by protozoan parasites of the *Leishmania* genus. This disease is a major public health problem in the New World affecting people exposed in endemic regions. The city of Governador Valadares (Minas Gerais/Brazil) is a re-emerging area for the visceral and tegumentary leishmaniasis, with 127 human cases reported from 2007 to 2013, and a lethality rate of 16%. The transmission of the disease occurs intensely in this region and more than 30% of domestic dogs present positive serology for the visceral form. Despite this scenario, little is known about the circulating *Leishmania* species in the insect vector *Lutzomyia longipalpis* in this focus. To address this, we conducted the collection of 616 female sandflies in different areas of the Governador Valadares city between January and September 2015. After DNA extraction of individual sandflies, the natural *Leishmania* infections in *Lu. longipalpis* were detected by end-point PCR, using primers derived from a kDNA sequence, specific to *Leishmania* genus. The sensitivity of this PCR reaction was 10 fg of *Leishmania* DNA and the infection rate of sandflies with the parasite was approximately 13%, totalizing 80 positives. Using species-specific primers, we detected the presence of multiple *Leishmania* species in *Lu. longipalpis* in Governador Valadares, including 3 sandflies infected with *L. amazonensis*, 28 with *L. infantum*, 5 co-infections of *L. infantum* and *L.* (*Viannia*) spp. and 44 infected with unidentified *L.* (*Leishmania*) spp. Our results suggest a role of *Lu. longipalpis* in the transmission of both tegumentary and visceral leishmaniasis in Governador Valadares and reveal a potential increasing risk of transmission of the different circulating parasite species. This information needs to be considered for surveillance and more effective control efforts against leishmaniasis in this endemic focus. **Supported by:**CNPq, FAPEMIG and CAPES **Keywords:**Sandflies; leishmania infection; pcr detection

PV087 - **METAGENOMIC ANALYSIS OF A PANTRONGILUS MEGISTUS GUT NATURALLY INFECTED WITH T. CRUZI USING NGS TECHNOLOGY**

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The Trypanosoma cruzi is the causal agent of Chagas disease. Its life cycle includes an invertebrate host, represented by an insects of the Reduviidae family, and a mammal. Chagas disease is a public health problem, infecting 10-15 million people in several countries in Latin America and the Caribbean, with approximately 14,000 people dying every year. The biology of T. cruzi and the relations with the human host are intensely studied. However, the study of the development of T. cruzi and the factors that regulate it in the insect vector is equally relevant. In recent years the microbiome, defined as the set of organisms that colonize a particular environment or host and how they influence it, has been the focus of many studies. In this sense, this work studied the intestine of the insect vector for Chagas disease of the species Panstrongylus megistus infected with T. cruzi, collected from nature in Santa Catarina - Brazil. The insect gut was removed and the total DNA purified and sequenced on the Illumina MiSeq and Ion Torrent (PGM) platforms. The gene encoding the 16S rRNA was amplified from the total DNA and also sequenced on the Illumina MiSeq platform. In total, 11 million sequences of total DNA and 1,831 sequences of 16S rRNA were obtained. Using the MG-Rast webserver, the sequences were analyzed for diversity with the biological databases Greengenes, RDP and SILVA SSU, observing the predominance of the phyla Proteobacteria. Functional analysis with the COG, SEED Subsystems and KO databases revealed sequences related to human diseases, including Chagas disease. The total DNA sequences were mapped on the T. cruzi reference, genomes available in the GenBank database, and around 8% of the sequences belongs to T. cruzi. These reads were assembled with the CLC Genomics Workbench yielding 18,500 contigs. This approach allowed the partial genome reconstruction of this particular T. cruzi specimen, as well the evaluation of its microbiome. **Supported by:**ICC - FIOCRUZ/PR
Keywords: Trypanosoma cruzi; panstrongylus megistus; microbiome

PV088 - **2-THIOCIANYL- 1,4-NAPHTHOQUINONE: A DRUG WITH TRYPANOCIDAL ACTIVITY THAT SELECTIVELY IMPAIRS THE VIABILITY AND GROWTH AT ALL STAGES OF THE *TRYPANOSOMA CRUZI* LIFE CYCLE.**

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Introduction: Chagas disease is a debilitate disease and it still does not have an effective cure at the chronicle stage. Considering the various factors such as (a) drug ineffectiveness to the chronic phase, (b) genetic diversity of the species with a varied virulence profile, (c) varying levels of drug resistance, it is necessary to search for new therapeutic agents to guarantee a better quality of life and a higher rate of cure for the patients, especially in its chronic phase. In this context, naphthoquinones appear as drug candidates, has been proposed that naftoquinones generate reactive oxygen species in the intracellular environment promoting oxidative stress with consequent cell death. Objective: Our goal in this study was to determine the trypanocidal activity of naphthoquinone, 2-Thiocianyl-1,4-Naphthoquinone (2TIONQ) using in vitro and in vivo assays. Methods: Investigation of cell proliferation of epimastigotes by flow cytometer using stain cell stability 7-AAD, cell viability with culture trypomastigotes or diverse mammalian cell lines using MTT assays to determine the inhibitory concentration (IC50) of 2TIONQ, besides in vitro infection with Vero cells to determine the endocytic/infection indexes. Biochemical tests of serum markers were perfomed in C57BL/6J mice in order to verify the toxicity in rodents. Results: Assays in epimastigotes indicated trypanocidal effect with significative IC50 of 3.27μM ± 2.2. In addition, the Vero, HeLa and Raw strains showed values that demonstrate selective toxicity of compound. Oral administration in murine of 0.2 mmol/kg and 0.4 mmol/kg did not show alterations in body weight, relative body weight and changes in serum markers, suggesting that 2TIO-NQ does not promote hepatoxicity and nephrotoxicity. Soon we will investigate whether the drug can inhibit the in vitro infection, which could put 2-Thiocyanil-1,4-naphthoquinone as promising candidate for chemotherapy in experimental Chagas' disease. **Supported by:**CNPq-SETEC, IFRJ-Programa PIBICT/Prociência
Keywords: Trypanocidal drugs; naphthoquinones; chagas`s disease

PV089 - **EUKARYOTIC EVOLUTION IN THE NEOPROTEROZOIC ILLUMINATED BY PHYLOGENOMICS AND ANCESTRAL STATE RECONSTRUCTION OF ARCELLINID SHELLS.**

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Shelled microbes are central tools in understanding the evolution of deep eukaryotic lineages, because these organisms leave a consistent and well-documented fossil record. However, thorough analysis of these fossil taxa is hampered by a lack of robust phylogenetic frameworks. The Arcellinida, a species-rich lineage in Amoebozoa, is one to have a number of Neoproterozoic fossil taxa associated with. Here we combine modern phylogenomic methods to generate a robust phylogenetic framework for the Arcellinida with ancestral state reconstruction methods to make inferences on the early evolution of shelled eukaryotes. We have generated transcriptome sequences from 20 arcellinid taxa, that represent the major lineages in the group, and analyzed this dataset through a phylogenomic pipeline to construct a tree based on ~350 genes and 100,000 amino acid sites. The resulting tree revealed a monophyletic Arcellinida, with 9 well-defined deep lineages, of which only two had been previously identified. The ancestral state reconstruction for the hypothetical ancestors of these nine lineages yielded morphologies that are strikingly similar to microfossils described in the Neoproterozoic for the Chuar Group (750mya) and the Bocaina formation (~830mya). These findings demonstrate that arcellinids diversified quickly after the major event of ocean oxygenation (850mya), and major lineages were well established before the great glaciations of the Cryogenian period.
Supported by: Fapesp **Keywords:**Amoebozoa; evolution; fossils

PV090 - **PRODUCTION AND ANALYSIS OF A SPECIFIC POLYCLONAL ANTIBODY AGAINST *TRYPANOSOMA EVANSI***

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Trypanosoma evansi is the etiological agent of the disease known as or Surra and is responsible for large economic losses in livestock. This work presents the development of antibodies against T. evansi to be used in diagnostics techiniques. Antisera were produced by the immunization of two Wistar rats. Rat 1 was immunized with fixed T. evansi formaldehyde and complete Freund's adjuvant. Rat control was immunized with PBS and complete Freund's adjuvant. The immunization procedure was extended for 52 days, involving four injections (one preparative dose of immunogen in complete Freund's adjuvant and three doses with immunogen in incomplete Freund's adjuvant). At the end of this period the animals were exsanguinated to obtain the serum. To evaluate the presence of antibodies, western blot were performed at different dilutions between 1: 500 and 1: 8000; and also tested for immunofluorescence by flow cytometry. In the western blot all the dilutions tested were positive and the serum of the negative animal showed no reaction. For flow cytometry the T. evansi purified by ion exchange chromatography on DEAE-cellulose was used. The analysis of antibody binding to the parasite was performed following steps of incubation with the serum and FITC-conjugated secondary antibody (Anti-Rat IgG-FITC). In flow cytometry, the parasites were resuspended in FACS buffer (1% BSA; PBS pH 7.4; 0.1% sodium azide) followed by the addition of propidium iodide (PI) for evaluation of cellular viability. The cells were then analyzed using a BD Accuri C6 flow cytometer (BD). The gating threshold for PI staining was determined using cells without PI staining. The percentages of cells that were stained positively for antibody were determined by the integration of cells above specified fluorescence channel, calculated in relation to negative control. The antibodies produced were reactive against T. evansi protein extract and in vivo. **Supported by:**CNPq
Keywords: Trypanosona evansi; antibody; flow cytometry

PV091 - **SEROLOGICAL ANALYSIS OF *MUS MUSCULUS T. EVANSI*- INFECTED USING THROUGH PROTEIN DEPLETION**

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Trypanosoma evansi, the agent of surra, is a salivarian trypanosome, responsible for losses in livestock. The differential diagnosis of trypanosomes is essential for the preventive and curative measures of the disease. The use of biomarkers, such as blood proteins is a powerful tool for the detection of a biological condition through the analysis of biological tissues or fluids. To identify potential biomarkers for detection of T. evansi infection, mice abundant serum protein were depleted from each sample using ProteoSpintm kit. To detect differentially-expressed proteins we applied a LC-MS/MS-based proteomics approach. Peak picking and protein identification, quantification and validation were performed on a MaxQuant platform. Low-abundant proteins from acute infection group sera (4 days) were compared to those from low-abundant proteins from uninfected control group sera. The analyzed proteins (168 in total) were classified according to their biological function in six groups: Cellular communication (CC), Cell growth and/or maintenance (CG), Immune response (IR), Metabolism (energy) (MT), Protein Fate (PF) and Transport facilitation (TF) where 6% were expressed exclusively in the acute infection group and 9% exclusively in the control group. Proteins were increased in 61% and decreased in 39% in the group of acute infection. Proteins that decreased the intensity had an average loss of 59% of their original values and those that increase the intensity were an average gain of 457%. The functional group CC is highlighted due a significant gain of 1547% comparing to other groups CG (74%), IR (379%), MT (545%), PF (92%) and TF (107%). The Psap protein of the CC group was noted for having its intensity increased by 4545%. Our experiments indicate that T. evansi infection in acute phase considerable increase proteins of all the selected groups. Nevertheless this is the first preliminary report of sera protein analysis of *T. evansi* acute infection in mice. **Supported by:**CNPq
Keywords: Trypanosoma evansi; low abundant proteins; mass spectrometry

PV092 - **EFFECT OF THE OVEREXPRESSION OF THE EIF4E5 TRANSLATION INITIATION FACTOR ON THE MORPHOLOGY, VIABILITY AND CELL GROWTH OF LEISHMANIA INFANTUM**

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In trypanosomatids the gene expression profile reveals that most genes are regulated predominantly at the post-transcriptional level, during mRNA processing, transport, decay and translation. In eukaryotes, the initiation stage of translation is the most regulated event during protein synthesis and requires the action of several eukaryotic Initiation Factors (eIFs). Paramount among these is the eIF4F complex, formed by three subunits (eIF4A, eIF4E and eIF4G). Six eIF4E homologues have been described in trypanosomatids, with two of those, EIF4E5 and EIF4E6, not yet properly studied in Leishmania. This work aimed to study EIF4E5 and investigate its importance for the viability and cellular morphology when altering its expression in L. infantum. The wild-type EIF4E5 was cloned and its gene used for transfection into L. infantum and expression as an HA-tagged fusion. Generation of single and double knockouts of the endogenous gene was also attempted. Viability and cell growth were analyzed by means of growth curves before and after gene complementation assays. The subcellular localization of eIF4E5 was done by means of immunofluorescence and the morphological analysis was performed through confocal microscopy. It was seen that the expression of the eIF4E5 occurs at every stage of cell growth and that it localizes to the cytoplasm. The overexpression of eIF4E5 in wild-type and single knockout backgrounds led to cells with morphological alterations, where a shortened size was seen when compared to the non-transfected L. infantum, indicating that EIF4E5 might be involved in regulating the synthesis of cytoskeletal proteins. Double knockouts of the EIF4E5 gene were not achieved, even when it was performed in the presence of a plasmid encoded protein, indicating that the factor may be essential for cell viability, but requiring further investigation. EIF4E5 emerges as a critical factor with functions associated with regulation of translation in Leishmania. **Supported by:**CAPES
Keywords: Translation initiation factors; trypanosomatids; eif4e

PV093 - **REACTIVE NITROGEN SPECIES AFFECTS *RHODNIUS PROLIXUS* IMMUNE RESPONSES, BACTERIAL MICROBIOTA AND *TRYPANOSOMA CRUZI* INFECTION**

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Many factors can affect the development of *Trypanosoma cruzi* in the invertebrate host, mainly immune responses such as the production of reactive nitrogen species and interaction with the insect´s intestinal microbiota. Herein, we investigate the effects of reactive nitrogen species in the insect vector, *R. prolixus*, on the infection of *T. cruzi*, the intestinal bacterial microbiota and signaling of other insect immune responses. Therefore, we treated fifth instar nymphs of *R. prolixu*s orally with L-arginine as substrate for production of reactive nitrogen species and L-NAME, analogue compound that inhibits the action of nitric oxide synthase. As results, L-arginine increased nitric oxide production and the transcript abundance of NOS encoding cDNA. Morevoer the L-arginine treatment reduced the cathalase activity and the antibacterial activity against *Serratia marcescens*, resulting in increasead amount of bacteria and low number of trypanosomes in the digestive tract. In contrast, the treatment with L-NAME, reduced the production of nitric oxide and the abundance of NOS encoding cDNA, increased cathalase activity, and increased the parasite number in digestive tract. We suggest that nitric oxide plays a fundamental role in insect immunity modulation, influencing the microbiota and the *T. cruzi* infection in the insect vector. **Supported by:**CAPES, FAPERJ, CNPq
Keywords: Reactive nitrogen species; rhodnius prolixus; trypanosoma cruzi

PV094 - **THE *IN VIVO* TRANSMISSION BLOCKING POTENTIAL OF MEFAS, A HYBRID COMPOUND DERIVED FROM MEFLOQUINE AND ARTESUNATE**

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Previous efforts to eradicate malaria have failed, due to the emergence of drug-resistant parasites, insecticide-resistant mosquitoes and the lack of drugs or vaccines to block parasite transmission. Now, one of the goals of malaria control is to prevent the parasite transmission to vector mosquitoes. Since most drugs target only the asexual stages of the parasite without reducing gametocytemia, the development of drugs to target the transmissible gametocytes are urgent for the effective control of malaria and for limiting the spread of drug resistance. Artemisinin and its derivatives are the current first-line antimalarials effective against the blood stage parasites and gametocytes, but resistance to its derivatives has now emerged and spread in various malaria endemic areas. A novel antimalarial drug or a new drug combination is needed to overcome this problem. The intense blood schizonticidal and gametocytocidal activities of MEFAS, a synthetic hybrid salt derived from mefloquine (MQ) and artesunate (AS), have been previously reported. The objective of this study was to evaluate the efficacy of MEFAS, in parallel with MQ and AS, and with the combination of AS+MQ at the same doses, on their ability to block transmission of the avian malaria parasite *Plasmodium gallinaceum* of the domestic chickens (*Gallus gallus domesticus*) to the vector *Aedes fluviatilis*. The results showed that MEFAS was effective on blocking the transmission of *P. gallinaceum*, like AS and the combination (AS+MQ). In conclusion, MEFAS was effective as an antimalarial able to block transmission in avian malaria. Whether it could be used safely against human malaria as a dual-acting antimalarial, able in cure infection and block the parasite transmission is now under investigation. The *ex vivo* tests of MEFAS activity are being performed with blood isolated from patients in the endemic area with *P. vivax* or *P. falciparum* in *Anopheles aquasalis* mosquitoes in Porto Velho, Western Brazilian. **Supported by:**CAPES
Keywords: Antimalarials; block transmission; drug resistance

PV095 - **CHARACTERIZATION OF ASSOCIATION BETWEEN TWO DISTINCT RBPS AND THE THREE POLY-A BINDING PROTEIN (PABP) HOMOLOGUES IN *LEISHMANIA***

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The Poly-A Binding Protein (Poly-A) is a major RNA binding protein known to participate during protein synthesis in eukaryotes. During translation initiation PABP binds to the initiation complex eIF4F, leading to the circularization of the mRNA and enhancing its translation. A typical PABP is formed by three distinct segments: the N-terminal RNA binding region, with four RNA-binding domains (RRMs); a non-structured linker segment; and the C-terminal MLLE domain. Trypanosomatids are characterized by unique processes associated with their gene expression and mRNA translation is no exception. Multiple eIF4F-like complexes have been characterized with likely distinct roles in translation. Three PABP homologues have also been identified, with likely distinct roles that need to be better defined. In previous co-precipitation experiments carried out with the aim of better defining the function of the three Leishmania PABPs, several RNA-binding proteins (RBPs), with typical RRMs, came down with the different PABPs and may be associated with their function. These include the proteins known as RBP23 and DRBD2. Here we investigate their ability to directly bind to the PABP homologues. This was performed through pull-down assays between the GST-tagged PABPs expressed in Escherichia coli and the 35S-labeled RBP23 and DRBD2. Both proteins bound efficiently to all three PABP homologues but the interaction with PABP1 was stronger. To map their binding site within PABP1, different GST-tagged truncated constructs were generated consisting of the PABP RRMs12, RRMs34, the MLLE domain and a C-terminal segment encompassing RRM4, linker segment and MLLE. The 35S-RBP23 bound best to the C-terminal segment while the 35S-DRBD2 had a preference for the RRM34 construct. None of the two RBPs bound to the MLLE domain only, shown to bind EIF4E4, a known PABP1 partner. These experiments are expected to advance the knowledge regarding the different RBPs of Leishmania and their role in translation. **Keywords:**Rna binding protein; rbp23; pabp1

PV096 - **BIOLOGICAL ACTIVITY OF RUTACEA FAMILY ESSENTIAL OILS IN *PHYTOMONAS SERPENS***

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The Phytomonas genus groups the plant-infecting heteroxenous trypanosomes from the Trypanosomatidae family. This genus is responsible for significant economic losses because they cause diseases in important agricultural products, for example coffee, tomatoes and palms. The removal of infected plants and use of toxic insecticides are the only control strategies. Essential oils are well known as an alternative microorganism control method. In this context we studied the biological effects of an essential oil, extracted from plants of the rutaceae family, that has sabineno, 2-undecanona e limoneno as major components. The plant was collected at the national park of Restinga de Jurubatiba/RJ. On two separate experiments we analyzed the oil activity in three concentrations and the influence of dimethyl sulfoxide, used for dilution. The oil biological activity was measured by counting the number of viable parasites on a Neubauer chamber through 7 days. The experimental tubes had the same medium and were kept at the same conditions used for culture. The cells were considered viable if they had movement and morphology similar to the control group. We found the IC50 to be 25µL/mL. Seen under microscope treated cells had morphological changes suggesting a process of cell death, aside from reduced mobility and reproduction. Our data shows that the essential oil from the rutaceae family has trypanosomicide agents that act inhibiting the parasites growth. Although the active compound has yet to be identified, further research of the oil is likely to result in less toxic and more efficient drugs against phytoparasitosis. **Supported by:**CNPQ
Keywords: Phytomonas serpens; essencial oil; control

PV097 - ***LEISHMANIA TARENTOLAE* AS EXPRESSION SYSTEM OF P24 ANTIGEN FROM HIV1**

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The Human Immunodeficiency Syndrome is one of the most infectious diseases in the world, representing a global challenge. The etiologic agent is the Human Immunodeficiency Virus (HIV), a retrovirus, transmitted mainly by sexual contact. The diagnosis has advanced greatly by the use of recombinant antigens for recognizing antibodies produced by infected patients. One antigen of HIV important for diagnosis is the nucleocapsid p24 protein. This protein is detected early after infection and remain in the bloodstream for a long period of time. Many kits for serological HIV identification using human samples are expensive because to use them it´s necessary pay taxes of royalties and importation. A second and important point is that the production of viral antigens in prokaryotic cells in many situations is not successful, justifying the development of new studies using alternative expression systems for obtaining viral protein in large quantities quickly and effectively. The objective of this study is express and purify the protein p24 of HIV1 using the expression system Leishmania tarentolae. The p24 protein of HIV 1 gene was amplified by PCR, cloning into pGEM-T-Easy and subcloning into pLEXSY hyg2. The plasmids were used to transfect Leishmania tarentolae and two transgenic lines have produced: a episomal expression and constitutive expression for p24 protein. For assessment of protein expression, cell extracts were fractionated in SDS PAGE 20% which allowed the visualization of protein expressed in the predicted size, the protein was purified using Nickelâ€‹ membrane and confirmed by Western blot assay using antibodies anti-p24. In conclusion, this work has achieved the expression of the recombinant protein p24 of HIV 1 in Leishmania tarentolae, and this new expression system can be used for expression of other viral antigens.
Supported by:CAPES **Keywords:**L. tarentolae; heterologous expression; p24 hiv

PV098 - **EVALUATING THE EFFECT OF OVEREXPRESSING TWO CAP BINDING PROTEIN HOMOLOGUES, EIF4E3 AND EIF4E4, ON THE TRANSLATION OF A REPORTER MRNA IN *TRYPANOSOMA BRUCEI***

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In eukaryotes, the trimeric eIF4F complex (formed by the eIF4A, eIF4G and eIF4E subunits) plays a relevant role in mRNAs recognition and facilitates the recruitment of ribosomes during translation initiation. In trypanosomatids, multiple homologues for the eIF4F subunits were identified but the specific functions for each of them still need to be better defined. Of the six homologues of eIF4E, the cap binding protein, previous studies have implicated only two in translation, EIF4E3 and EIF4E4. The complex formed by EIF4E4 and its partner EIF4G3 is the most probable eIF4F complex acting in translation initiation. Another eIF4F-like complex is formed by EIF4E3 and EIF4G4. This study aimed to evaluate the effect of overexpression of EIF4E3 and EIF4E4, and mutants impaired in their ability to bind to their eIF4G partners, on the translation of a reporter mRNA encoding the green fluorescent protein (GFP) through a tethering assay. For that, we generated cell lines of procyclic Trypanosoma brucei expressing different reporter mRNAs harboring or not a hairpin in the 3'UTR (BoxB). Those cells were then transfected with plasmids for inducible overexpression of EIF4E3 and EIF4E4 (or their mutants) in fusion to the λN-peptide, which binds to BoxB. Confirmation of EIF4E3 and EIF4E4 overexpression was performed by Western blot and cell growth were evaluated by cell counting. GFP expression was assessed by flow cytometry and fluorescence microscopy. The reporter cell lines were shown to be feasible to use in the characterization of proteins involved in control of gene expression in T. brucei. In general, the overexpression of these proteins did not interfere with cell growth; however, the mutation in the eIF4G binding site seems to reduce stability of the EIF4E4 protein. When tethered to the mRNA, only EIF4E3 had a major effect on GFP levels, mostly by increasing its mRNA stability. These results validates the assay and implicates further EIF4E3 in regulating mRNA expression. **Supported by:**CAPES
Keywords: Eif4e; trypanosomatids; translation

PV099 - **COMPLEMENTATION ASSAYS IN *LEISHMANIA INFANTUM* DEFINE MAJOR FUNCTIONAL DIFFERENCES BETWEEN TWO HOMOLOGUES OF THE POLY-A BINDING PROTEIN (PABP)**

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Poly-A binding proteins (PABPs) are abundant and conserved eukaryotic proteins with critical roles in the control of mRNA translation and stability, among other functions. In trypanosomatids, it is assumed that PABP homologues may have relevant roles as well and in Leishmania three PABP homologues have been identified. PABP1 seems to have a more general role in translation whereas PABP2 and 3 interact with each other and appear to be associated with distinct mRNA populations. Previous work has demonstrated the essentiality of PABPs 1 and 2, but not PABP3 in *Leishmania*. In order to better understand their function, this work aimed to investigate the ability of PABP1 and PABP2 mutants to complement the absence of their respective native proteins in *L. infantum* promastigote forms. For this, the deletion of both copies of the endogenous PABP 1 and 2 genes (double knockout - DKO) was carried out in the presence of an ectopic gene encoding a HA-tagged fusion protein. The double knockout is only possible in the presence of either the wild-type protein or mutants that do not have their function impaired. Different mutants were generated for PABPs 1 and 2 to investigate motifs potentially important for their function. All mutations replaced three consecutive residues for alanines. The mutants were transfected into Leishmania cells with a previous deletion of the first copy of the wild-type gene, followed by an attempt to delete the second copy and an evaluation of the cell survival in the presence of the mutant protein. Viable cells were recovered after complementation with all PABP2 mutants tested, with the exception of the TGM mutant targeting the protein C-terminal MLLE domain. For PABP1, viable cells were recovered with all mutants, but wild-type gene duplication occurred for cells having the LMW and YGF mutants, on the protein's N-terminal RNA binding region. These results are consistent with the two proteins having major functional differences yet to be defined. **Supported by:**CNPq, Capes
Keywords: Translation initiation; regulation; protein synthesis

PV0100 - **MEASUREMENT OF PHOSPHATIDYLSERINE EXPOSURE BY *L. AMAZONENSIS* STRAINS SUBMITTED TO SUCCESSIVE *IN VITRO* PASSAGES.**

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It is well known that successive *in vitro* passages (SIVP) lead to attenuation of virulence. However, it is not well know about the metabolism and the pathways involved in loss of virulence after SIVP. The exposure of Phosphatidylserine (PS) is a very important mechanism used for opsonization and parasite surviving inside the parasitoforus vacuole in macrophages. In an attempt to elucidate these questions we have previously performed a metabolomic multiplatform fingerprint approach (GC/MS, LC/MS and CE/MS) comparing *L. amazonensis* axenic promastigotes after 60, 40 and 10 successive in vitro passages to wild strain. Among the several metabolites (n=67), PS was found in a decrease concentration in the attenuated strains metabolome. Here, PS exposure was measured by flow cytometry assay. In addition, the SIVP strains were used to infect macrophages derived from bone marrow of BALB/c bone marrow macrophages (WT) and Balb/c CD300a knockout mice, were compared. The family of CD300 molecules modulates a wide and diverse range of processes in immune system cells, highlighting the pathway activation of pairing and inhibition of receptor functions. These molecules are able to recognize extracellular lipids, such as ceramide, phosphatidylserine and phosphatidylethanolamine. The flow cytometer analyzes demonstrated decreased exposure of PS in attenuated strains, as compared to the wild type strain, in agreement to the metaboloma profile. Although the attenuated strains are less infective to WT macrophages, there were no significant differences in percentage of infected macrophages by comparing WT and CD300aKO cells, for each of the strains tested. This result suggests that although SIVP affects PS metabolism and its exposure in cell surface, the infectivity differences among the SIVP strains may not be solely attributed to the PS exposure, since deficiency of CD300a receptors did not altered their infectivity pattern for macrophages.
Supported by:CNPq **Keywords:**Metabolomics; leishmania amazonensis; phosphatidylserine exposure

PV0101 - **PARALOG RNA-BINDING PROTEINS DUPLICATION RENDER DISTINCT POSTTRANSCRIPTIONAL REGULATORY NETWORKS IN *TRYPANOSOMA CRUZI***

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A key-step of cell adaptation is the ability to exert a tight control of gene expression. One main issue in biology is how regulatory proteins evolve so that they generate new gene networks.Posttranscriptional regulatory networks define RNA regulons, where functionally related transcripts are co-regulated on a particular ribonucleoprotein particle (RNP). RNA binding proteins (RBPs), which recognize specific elements on transcripts, orchestrate this regulation. Gene duplication may lead to different outcomes, due to a relaxation of selective pressures acting on one isoform. Gene duplication of RBPs is common in Trypanosomatids. However, there is little information on how these duplications impact in gene regulatory networks. TcRBP40, a previously characterized RBP from *Trypanosoma cruzi*, is a paralog of TcRBP7, a duplication that is also present in other related species. Sharing 73% identity, they have a conserved tridimensional structure on its RNA Recognition Motif, show similar expression patterns and co-localize in *T. cruzi* epimastigotes' posterior vacuoles, suggesting functional redundancy. However, immunoprecipitation assays and RNA-Seq of their associated mRNAs, showed that they associate to distinct transcript sets. TcRBP40 is found associated mainly to mRNAs coding for MASPs, and TcRBP7 not presenting a significant particular functional group. Their putative recognition elements on the transcripts are similar, but both proteins show differences in their ability to recognize and bind these elements. Our results suggest that mild changes in sequence or structure of regulatory RBPs might be sufficient for reprogramming a gene regulatory network. **Supported by:**CNPq, Fiocruz
Keywords: Gene duplication; rbp; rna regulon

PV0102 - **SUCCESSIVE *IN VITRO* PASSAGES TRIGGERS CHANGES IN THE PROTEOMIC AND METABOLOMIC PROFILES OF *L. AMAZONENSIS*, LEADING TO ATTENUATION OF VIRULENCE.**

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It is well known that successive in vitro passages (SIVP) lead to attenuation of Leishmania and has been used for virulence studies. In an attempt to elucidate this process, our group performed a metabolomic assay (Met), comparing a virulent L. amazonensis wild type (R0) with its attenuated strains after 10, 40 and 60 SIVP. Met identified significant differences (SD) in 67 metabolites belonging to 8 enriched pathways, associated to lipid biosynthesis(LBP) and redox system (RDx). Nevertheless, prior to our study, a published proteomic approach (Prot) also compared R0 with its attenuated strains after 10, 20 and 30 SIVP, demonstrating SD in 58 proteins such as Trypanothione Reductase(TR), Tryparedoxin Peroxidase(TP), Malic Ezym(ME) and Isocitrate dehydrogenase NADP mitochondrial precursor(ID-NADP). All together, these data allowed the construction of a biochemical map(BM) highlighting the metabolic pathways(MP) and metabolites changes during the attenuation process after SIVP. In order, to validate the BM, in vivo assays were performed. The evidence of RDx participation were determinate by a trivalent antimony (SBIII) resistance test(EC50) and qPCR analyses for TR and TP encoded genes. SD at EC50 levels were observed, by comparing SIVP strains to R0, which were more sensitive to SBIII. The qPCR analysis showed that transcripts for TR and TP were increased in SIVP strains, a similar pattern of expression demonstrated in Prot analyzes. The participation of LBP was confirmed by the exposure of phosphatidylserine(PS) by flow cytometry assay. Decreased exposure of PS were observed in SIVP strains, in agreement with the lower abundance of intracellular PS in Met. Transcript levels for ME and ID-NADP also agreed with the levels of expression found in Prot. Therefore, these results corroborated and revealed that the Met and Prot data complementarity, which demonstrated that this association can be useful to validate the proposed BM of the Leishmania attenuation process.
Keywords: Metabolomics; leishmania; virulence

PV0103 - **DEVELOPMENT OF A DUPLEX REAL-TIME PCR TEST, FAST AND READY-TO-USE TO DETECT CYCLOSPORA CAYTANESE IN CONTAMINATED FOOD USING REAGENTS PRODUCED IN BRAZIL**

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Ciclospora cayetanensis is a protozoan that causes a gut disease called cyclosporiasis, which is associated with the consumption of C. Caytanesis oocystis on contaminated food and water. Usually, outbreaks of cyclosporiasis in the United States are caused by contaminated imported fresh foods, such as raspberry and cilantro. Therefore, we developed a real-time PCR-based diagnostic method in gelified format for the quick detection of C. caytanesis. An internal amplification control (IAC) was designed from the genomic sequence of C. caytanesis. We performed a dilution curve starting at 104 oocysts to determine the limit of detection of the test. A single laboratory validation method was used to evaluate the method of detection with samples donated by the Food and Drug Administration (FDA). These samples consisted on cilantro and raspberry samples, contaminated with 0, 5, 10 and 200 oocysts of C. caytanesis. It was possible to detect 0.5 oocysts per microliter and 2.5 oocysts per reaction (10-1), with 100% efficiency, in gelified format. The detection rates of samples contaminated with 5 and 10 oocysts were respectively 31.25% and 87.5% for cilantro, and 25% and 50% for raspberries. All samples without the presence of oocysts were negative for both contaminated foods. All samples contaminated with 200 oocysts were positive. The gelified format consists on a robust method, that is simple and does not compromise the sensitivity and precision of the test. It contributes to adequate clinical management of patients, provide rapid disease detection, aiding epidemiological control at times of disease outbreaks, and can detect C. caytanesis oocysts in a simple and rapid way in contaminated food.
Keywords: Cyclospora caytanesis; ready to use; real time pcr