VE001 - **THE LOCOMOTOR ACTIVITY OF RHODNIUS PROLIXUS IS DIFFERENTLY AFECTED BY INFECTION OF TRYPANOSOMA CRUZI OR TRYPANOSOMA RANGELI**

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Triatomines are nocturnal insects, and during daytime they stay aggregated in akinesis, i.e. without moving, inside shelters. However, triatomine activity is strongly increased in two phases of their daily cycle. One activity peak related to the search for hosts is observed at dusk. The second peak related to refuge search precedes dawn. Using ad hoc developed actometers, we measured the locomotor activity of fifth instar *Rhodnius prolixus* previously infected with either *Trypanosoma cruzi*, *T. rangeli* or not infected. During assays, the movement of insects inside arenas interrupted light beams, generating pulse signals sent to a computer for storage. Results showed that all insects, infected or not, showed the typical activity pattern known for triatomines. However, *T. rangeli* infected insects showed a general increase in locomotor activity, when compared to those from the control group. Exceptionally, during peaks their activity was lower. Differently, infection with *T. cruzi* induced insects to be less active, especially during the scotophase. Results obtained with *T. rangeli* infected insects suggest that a higher exposure to predators might be expected. In a natural context, such differences in the dynamics of locomotor activity of infected triatomines may have implications on the transmission rates of both trypanosomes.

Supported by:FAPEMIG, CPqRR, INCT-EM

VE002 - **NATURAL TRANSMISSION MODEL AND DOSE QUANTIFICATION OF LEISHMANIA CHAGASI (SYN LEISHMANIA INFANTUM) TRANSMITTED BY THE BITE OF THE LUTZOMYIA LONGIPALPIS, VECTOR OF AMERICAN VISCERAL LEISHMANIASIS**

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This study presents for the first time the establishment of a natural transmission model of L. chagasi by bite of female L. lonpipalpis, vector of American visceral leishmaniasis. During the blood meal, the sandfly can ingests parasites with the blood from the vertebrate host. Approximately 14 days after blood meal infection, the metacyclics, the infective forms to the vertebrate, appears. In the New World, the L. chagasi dose transmitted to the mammalian host has never been directly determined. In order to show that, we developed a natural model of transmission by bite of infected L. longipalpis. Experimental infection of 700 sandflies was performed with L. chagasi (MHOM/BR/70/BH46). The parasite load in the midgut was determined after infection. For natural transmission, 14-day infected sandflies were allowed to bite hamsters and Balb/C mice (each ear was exposed to one or two insects). Immediately after the bites, the animals were sacrificed and ears were processed for quantification of parasites transmitted into the ears. The DNA was extracted and the number of parasites determined by real time PCR. Our results of experimental infection demonstrated a large amount of parasites in the midgut of the vector (10,000), which persists even after the blood meal digestion. We concluded that these parasites were successfully transmitted by bites of infected sandflies. Hamster and mouse are good experimental models for laboratory natural transmission. The L. chagasi dose transmitted by the bite of a single L. longipalpis range from 10 to 10,000 parasites, however, 75% of the sandflies transmitted less than 300 parasites. This model is a powerful tool to study L. chagasi infection naturally transmitted by the vector.

Supported by:FIOCRUZ, FAPEMIG and CNPq

VE003 - **TRIATOMINE-TRYPANOSOMA INTERACTION: DO TRYPANOSOMA CRUZI AND TRYPANOSOMA RANGELI COLONIZE THE RHODNIUS PROLIXUS ANTERIOR MIDGUT?**

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After entering the triatomine gut with the blood meal, *Trypanosoma cruzi* and *T. rangeli* are confronted with changes on osmolarity, temperature, pH, presence of digestive enzymes and many other conditions of this new micro-environment. Regardless of the importance of the initial colonization phase, few studies had been developed about the establishment of the infection in the vector gut. In this study, the distribution of *T. cruzi* (CL strain) and *T. rangeli* (Choachi strain) in the *Rhodnius prolixus* anterior midgut was evaluated through different hours after infective meal on mice. Fifth instar nymphs were allowed to feed on infected mice, previously anesthetized, and, in order to estimate the number of ingested parasites, the insects were weighted before and immediately after feeding. Bugs anterior midguts were dissected in different periods post-infection and parasite quantification was determined with a Neubauer chamber. Giemsa stained slides were prepared in order to estimate the proportion of trypomastigote and epimastigote forms over time. For *T. rangeli*, the percentage of recovered parasites ranged around 60% from three hours to 7 days after infective meal. Fifteen days after infection, the number of recovered parasites decreased to 11%. Circular and epimastigote forms started to appear three hours after the infective meal, but trypomastigote forms were still observed until the 7th day post-infection. For *T. cruzi,* 100% of the parasites were recovered from the infective meal. However, this percentage decreased to 21 and 0.6 at 16 and 24h, respectively. Only trypomastigotes, some of them in a circular form, were observed for *T. cruzi.* These results indicate different mechanisms of establishment for *T. cruz*i and *T. rangeli* that could have implications on the parasite growth and development in the invertebrate host.
Supported by:FAPEMIG, CPqRR, INCT-EM

VE004 - **TEMPERATURE EFFECTS ON GROWTH OF TRYPANOSOMA CRUZI AND TRYPANOSOMA RANGELI IN VITRO**

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Environmental temperature can determine the course of an infection in parasite-insect interactions, including replication and virulence of the parasite. In this study, the proliferation of *T. cruzi* and *T. rangeli* in culture was evaluated at different temperatures (21, 24, 27 and 30°C) by flow cytometry. The parasites were grown in LIT medium supplemented with 15% fetal bovine serum, with an initial concentration of $1x10^6$ par/ml. They were maintained in temperature-controlled boxes for seven days. Daily, a sample of each culture was collected, labeled with the fluorescent dyes Fluorescein Diacetate and Propidium Iodide, and the numbers of living and dead cells were counted in a flow cytometer. Samples without dyes were used as controls. Growth of T. cruzi epimastigotes increased with increased temperature, with a 27-fold population increase at 30°C, by day 7. Mortality at this point ranged from 1 to 12% at 30 and 21°C, respectively. For T. rangeli, maximum growth was seen for parasites maintained at 27°C, when the population increased 7.5-fold. Mortality rates were around 5% at all temperature tested. In general, *T. cruzi* parasites reached higher populations than *T. rangeli* in an environment with the same nutritional supplies. These differences could be associated with variations in parasite size or nutritional requirements. Also, *T. rangeli* is apparently more sensitive to variations in temperature than *T. cruzi*. The next step is to evaluate how temperature influences parasite growth inside the vectors.
Supported by:FAPEMIG, CPqRR, INCT-EM, CNPq

VE005 - **ANALYSIS OF TRIATOMA BRASILIENSIS POPULATIONS USING THE MITOCHONDRIAL GENE CYTOCHROME OXIDASE 1**

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Triatoma brasiliensis s.l. is considered the main vector of Chagas disease in the Northeast Brazil. Recently, *T. brasiliensis* s.l. has been considered a complex formed by 4 specific taxa and one subspecies: *T. brasiliensis brasiliensis*, *T. brasiliensis macromelasoma*, *T. juazeirensis*, *T. sherlocki* and *T. melanica* that present distinct ecological behaviors and distinct chromatic patterns. Therefore, it is important to do a precise identification of the species which are dealing to ensure that the control actions are really effective. We analyzed the mitochondrial gene Cytochrome oxidase 1 (Cox1) sequences of 10 populations of *T. brasiliensis* from three Northeastern States, Pernambuco, Paraíba and Rio Grande do Norte, with the purpose of providing molecular data to establish differences and the degree of genetic relationship that occurs between these populations. Neighbor Joining (NJ) tree building methods was used to analyze the relationship between these species. The tree obtained showed great similarity between the populations studied with little more than 0.05 of distance and two major clades. The first one, basal, was established by the population of Salgueiro-PE with more than 0.04 in relation to other populations. It is likely that *T. b. macromelasoma* since this location is considered a type-location of this subspecies. The second clade, showed the population of Pernambuco more basal relative to the other, however, the population of Serra Talhada-PE formed cluster with populations of Paraíba, very likely due to the city is close to the border of Pernambuco and Paraíba. Populations of Paraíba formed a large cluster which also included the population of Rio Grande do Norte. The similarity of these populations may be related to the geographical proximity and similar environmental conditions. The inter-population divergence values (<0.01) may mean that these populations are still at the beginning of the speciation process. The results suggest that there is gene flow between populations that may involve transport liability associated with human movement in this region. These results corroborate with results obtained by other authors

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VE006 - **TRYPANOSOMA RANGELI INDUCES AN INCREASE IN THE RATES OF PREDATION ENDURED BY RHODNIUS PROLIXUS**

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Triatomines live inside shelters, where they aggregate with co-specifics during most of their lives. At dusk, they eventually leave the refuges searching for hosts and return to them mostly before dawn. As insect predators are commonly their hosts, triatomines can be killed during host search and the subsequent feeding process. In this study, we evaluated whether the infection by *T. rangeli* alters *R. prolixus* predation rates. Assays were performed in square glass arenas (40x40x20cm) presenting one central refuge of 10 cm² with two accesses. A mouse kept in a cage that allowed insect contact, was placed inside each arena at the start of assays. The percentage of insects that remained exposed outside shelters at the end of a 96h acclimatization period was 44 and 20% for infected and control groups, respectively. In the first two hours after host presentation, 43% of the infected insects had already left the shelter, while only 7.5% of the control insects were seen outside. In the presence of a host, both groups showed an intense shelter-related activity, i.e., leaving and entering shelters. However, a larger proportion of insects from the control group returned to shelters. This happened because infected insects were more predated by the hosts than not infected ones (61 and 47% for infected and control groups, respectively). One hour after lights on, approximately 5% of the insects from control groups were outside the shelters, while 27% of surviving infected insects remained exposed in the arena. These results confirm previous studies showing that the infection by *T. rangeli* alters shelter use by *R. prolixus*, inducing a higher exposure risk. The increase observed in the predation ratios imposed by mammal hosts may affect parasite transmission rates in nature.

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VE007 - **STUDY OF THE SPERMATHECA OF SIX TRIATOMINAE (HEMIPTERA, REDUVIIDAE)**

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By describing *Trypanosoma cruzi* in 1909, Carlos Chagas disclosed the epidemiological chain of the disease, which means 102 years have passed since the first vector, *Panstrongylus megistus* infected by the protozoan. After that observation, more and more studies have been carried out on triatomines, yet there are few researches on their internal morphology. In order to contribute to the expansion of such studies, the spermatheca of *P. megistus*, *P. herreri*, *Rhodnius neglectus*, *R. prolixus*, *Triatoma infestans* and *T. tibiamaculata* was observed. The specimens, which were obtained from the colonies kept at the Triatominae Insectary of the School of Pharmaceutical Sciences – UNESP – Araraquara, were sedated with chloroform, had the connexivum removed, and were immersed in physiological solution. Then the intestine was removed, a procedure that provided a better view of the reproductive organ to be studied under a Leica MZ APO stereoscopic microscope and the Motic Advanced 3.2 Plus image analysis system. The spermatheca of *P. herreri* has an elongated body, and its terminal portion is a little oval-shaped. In P. megistus the spermatheca presents a short loop and a more oval head than in *P. herreri*. Females of *Rhodnius neglectus* and *R. prolixus* showed elongated, loop-shaped spermatheca, with no differences when observed under optical microscopy. In *T. infestans* the spermatheca has a thin little insertion in the common oviduct, with a large oval body, whereas in *T. tibiamaculata* it presents a thin and elongated insertion and a thin body, just the terminal portion being somewhat oval. The observations showed that there are differences among the three genera regarding the spermatheca, as well as between *P. herreri* and *P. megistus*, and *T. infestans* and *T. tibiamaculata*. No differences were found in the spermathecae of *R. neglectus* and *R. prolixus*.

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VE008 - **ECTO-PTPASE ACTIVITY FROM TRYPANOSOMA RANGELI IS RELATED TO RHODNIUS PROLIXUS SALIVARY GLANDS INTERACTION**

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PTPases have been reported as a virulence factor in different pathogens. The physiological role of these enzymes in trypanosomatids had not been well established yet, although they are supposed to be involved in the virulence and cellular differentiation. We report here the presence of an ecto-PTPase activity in T. rangeli epimastigotes and the role of this enzyme during interaction between this parasite and R. prolixus salivary glands. In brief, salivary glands of R. prolixus were incubated in the presence of short and long epimastigotes forms of T. rangeli (106 parasites per salivary gland). Three different strains of T. rangeli were analyzed, namely strains H14, Choachi and Macias. The results demonstrated that long epimastigotes forms of H14 and Choachi strains exhibit higher levels of ecto-PTPase activity and adhesion capacity to salivary glands. Furthermore, short epimastigotes forms of all strains tested exhibit lower ecto-PTPase activity and adhesion to salivary glands compared to long epimastigotes. We also verified that orthovanadate, a PTPase inhibitor, could significantly inhibit T. rangeli adhesion to the salivary gland. The irreversible profile of PTPase inhibition produced by orthovanadate led us to study the effect of intracelomic infection by T. rangeli in R. prolixus, when ecto-PTPase activity was both fully functional and inhibited by pretreatment with orthovanadate. The inoculation of long epimastigotes pretreated for 1 h with different concentrations of orthovanadate impaired the T. rangeli infection in triatomid bug. Taken together, the results suggest that the ecto-PTPase activity from T. rangeli may play a role in the interaction with salivary glands of R. prolixus.

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VE009 - **STUDY OF THE INTERACTION BETWEEN TRYPANOSOMA RANGELI AND NITROPHORINS IN THE SALIVARY GLANDS OF THE TRIATOMINE RHODNIUS PROLIXUS**

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Trypanosoma rangeli develops in the intestinal tract of triatomines and invades the hemolymph and salivary glands (SG) of the insect vector, particularly in species of the genus *Rhodnius*. Among several bioactive molecules produced in the SG, the most abundant in *R. prolixus* are hemeproteins called nitrophorins (NPs). These proteins present a heme group and are capable to bind nitric oxide. In this work, we studied the interaction between *T. rangeli* and NPs, when the parasite is inside *R. prolixus* SG. The NPs 1-4 were knocked down by RNAi technique in *R. prolixus* nymphs, but the significant reduction in the NPs expression did not affect the survival and the differentiation process of parasites in the SG. As the absence of NPs does not interfere with the development of the parasite in the SG, we investigated whether the presence of *T. rangeli* parasites in the SG affects the salivary composition and the expression of NPs. *R. prolixus* fourth instar nymphs were inoculated in the hemolymph with 100 epimastigotes of *T. rangeli* in PBS, or with PBS only (control group). After molt, the SG of fifth instar nymphs starved for 40 days were disrupted in PBS. The RNA was extracted from gland tissue and the Real Time PCR demonstrated that expression of three housekeeping genes (18S rRNA, b-actin and a-tubulin) and the four NPs did not alter in SG after *T. rangeli* infection. The spectrophotometer quantification showed that the amount of total protein (280nm) and hemeprotein (404nm) were significantly higher in non-infected SG content. However, the proportion of hemeproteins/total protein was similar in both control and infected salivary contents. Based on these preliminary results, we conclude that *T. rangeli* did not affect the NPs transcription and translation and did not promote an expressive SG damage enough to reduce the expression of housekeeping genes in the SG. *T. rangeli* parasites probably only incorporated unspecifically the soluble proteins present in *R. prolixus* saliva. Supported by:FAPEMIG, CAPES, CPqRR, INCT-EM

VE010 - **MORPHO-PHYSIOLOGICAL DIFFERENCES BETWEEN THE INSECT ONCOPELTUS FASCIATUS NATURALLY INFECTED AND UNINFECTED WITH LEPTOMONAS WALLACEI**

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The insect *Oncopeltus fasciatus* belongs to the order Hemiptera and is a natural host of several species of trypanosomatids of the genera *Crithidia*, *Leptomonas* and *Phytomonas*. The colony of *O. fasciatus* that we maintain in our laboratory is naturally infected with *L. wallacei*. With the aim of obtaining insect-free *L. wallacei*, four hundred and fifty eggs at the same developmental phase were collected from the parental colony, treated in a solution of 2% sodium hypochlorite for 5 min, washed in sterile PBS and then dried on sterile filter paper. After the eggs ecloded, the insects were fed with peeled sunflower seeds and mineral water. After the eggs ecloded, on days 12 (third-stage nymphs), 17 (fourth stage nymphs), 22 (fifth stage nymphs) and 35 (adult insects), thirty insects were collected from each of the four groups and dissected for the extraction of the guts. After extraction, the guts were homogenized separately and the contents of each gut were analyzed under optical microscopy, for detecting the presence of flagellates. The visualization of at least one mobile parasite characterized the insects as infected by *L. wallacei*. The absence of protozoa in those samples was confirmed by PCR, using primers specific for *L. wallacei*. A colony of *O. fasciatus* free of trypanosomatids is being kept in other laboratory, far away from the original colony. The morpho-physiological analysis showed quantitative differences between insects naturally infected and uninfected with *L. wallacei*. Male and female adult insects from both colonies were comparatively analyzed with respect to weight, size, wing size, hemi-elytra size and fat body weight. Significant differences were observed in most of the criteria used, according to statistical analysis (ANOVA). The only exception was when we measured the weight of infected and uninfected male insects, where no significant difference was detected. Virgin insects were also analyzed in both groups; however, there were no significant differences among these insect groups, using the ANOVA test. Through egg load count inside the abdomen of infected and uninfected females, we observed higher production of eggs in the colony of insects free of *L. wallacei*, using Student's *t* test. The profile of ecloded eggs from infected and uninfected insects was also observed. In the uninfected colony, 85% of the laid eggs ecloded, whereas only 73% of the laid eggs ecloded in the infected colony. Insects from both groups were analyzed from ecloded eggs until adult insects; a deficit was observed in the development of infected insects in relation to the uninfected ones. We conclude that the infection of *O. fasciatus* by *L. wallacei* promotes a decrease in the fitness of these insects. The results presented here may be considered a model for the study of parasite-insect interactions in other ecological systems. Supported by:CNPq, FAPERJ, Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular (INCT-EM)

VE011 - **SEROCONVERSION OF CHICKENS NATURALLY EXPOSED TO LUTZOMYIA LONGIPALPIS, IN AN ENDEMIC AREA OF VL: POSSIBILITY OF THE CHICKEN AS A SENTINEL ANIMAL**

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Leishmaniasis is transmitted by *Lutzomyia longipalpis*, during the blood meal, when saliva and parasites are injected into host skin. The presence of chickens in endemic area is an important food and their presence is often cited as a risk factor to VL transmission. However the role chickens in the disease epidemiology has not been defined. In this sense antibodies could be used as an indicator of host exposure to the vector. Our aim is to detect antibodies against salivary gland sonicate (SGS) that could be used as exposure markers to VL and investigate the potential role of chickens as sentinels. Forty chickens were distributed in five houses in Cavunge, an endemic area in Bahia, and naturally exposed to sand flies during eight months. To evaluate vector density, light traps were distributed and monitored monthly in the same houses. Chicken blood was collected every two months for monitoring the seroconversion to SGS. ELISA was performed for the recognition of SGS and salivary recombinants proteins (rLJM17 and rLJM11). Sera from chickens experimentally immunized with SGS was used as positive control. Data were analyzed by statistical tests: Spearman, Kruskal-Wallis and Wilcoxon. Chickens pre exposed in endemic area showed anti-SGS titers (OD) varying from 0.001 to 0.025. After 4 months 26% of the exposed chickens became positive and 6 months later 100% were positive. After 8 months all of them were positive with high antibodies titers against SGS (0.124), 2.7x above *cut-off* value. Beside SGS we used rLJM17 and rLJM11 and these recombinants proteins showed similar performance as SGS. The correlation test was positive between specific antibody anti-SGS levels and sandfly density [r=0,5157 ($p \leq 0.01$)], which means the presence of sandfly in the field correlates with high levels of SGS antibodies in chickens.These results may be used as important indicators of the vector presence in endemics areas, raising the possibility of using the chicken as a sentinel animal.
Supported by:CNPQ

VE012 - **LEAVING THE DARK SIDE: INFECTION BY TRYPANOSOMA RANGELI WEAKENS THE NEGATIVE PHOTOTAXIS OF RHODNIUS PROLIXUS NYMPHS**

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Triatomines show a strong negative reaction to light, i.e. a negative phototaxis, which has a very low light detection threshold and is under circadian control. These characteristics are suggestive of a highly adaptive value, as they induce insects to search for refuges, avoiding their exposure to predators. Trypanosoma rangeli infects both mammals and triatomines and, although it is considered nonpathogenic to human hosts, it is known to induce different levels of pathogenicity in their invertebrate hosts, including behavioral alterations. The present study evaluated whether the infection by T. rangeli can modify the negative phototaxis presented by Rhodnius prolixus. For this, we used T. rangeli infected 5th instar nymphs starved for 30 days, while healthy insects were used for control tests. Assays were performed in a rectangular arena (14x4x2.5 cm) divided in two sectors, which allowed testing control and infected insects simultaneously in separate receptacles. Half of the arena was kept dark by means of a piece of black cardboard fixed over a glass cover and the other half remained illuminated (190 lux). The time spent at the darker sector and the number of passages between areas were evaluated during 10 min. The number of passages was similar for the two groups. However, insects from the control group spent 72% of the test period in the dark sector, while this was significantly reduced to 63% in infected insects. These results indicate that the negative phototaxis of infected insects is dampened, suggesting that they might increase their exposure at illuminated areas. In its turn, this might intensify the risk of being predated and, consequently, the transmission of parasites to mammals.
Supported by:FAPEMIG, CPqRR, INCT-EM

VE013 - **STUDY OF PROTEINS RELEASED BY TRYPANOSOMA RANGELI IN VITRO**

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The Chagas disease (Chagas, 1909, 1911) is an important parasitic illness caused by the protozoan Trypanosoma cruzi and transmitted by insects of the subfamily Triatominae. T. cruzi is not pathogenic for the invertebrate host, unlike Trypanosoma rangeli. The life cycle of T. rangeli in the invertebrate host begins when trypomastigote forms in the blood of infected vertebrates are ingested by the insect. They migrate to the midgut, cross the intestinal wall, fall into the hemolymph, invade salivary glands and can be inoculated in other vertebrates during the blood meal. The process of cell invasion in the insect is still poorly understood. Thus, our proposal is to detect, isolate and identify proteins released by T. rangeli in the process of the interaction with the gut of Rhodnius prolixus and investigate their importance in cell invasion. We used epimastigotes of T. rangeli, strain SC-58, grown at 28º C in liver infusion-tryptose medium supplemented with 10% fetal bovine serum and 0.4% hemin (stock of 2.5 mg/ml). Passage was done once a week with an inoculum of 20%. Gels were made with pure culture medium supplemented with 10% FBS or conditioned by the parasite for 10 days. Culture medium conditioned with T. rangeli for 10 days revealed the presence of molecules released by the parasite. One of these molecules appeared only after parasite presence in the medium and was of approximately 120 kDa and may correspond to a "porin like" rangelysin. Others molecules observed between 40 and 80 kDa may correspond to proteinases and lectins that may be involved in the case of intestinal invasion, during the vector-parasite interactions. We believe that identification of proteins released by the protozoa in culture may represent a promising avenue for understanding the pathogenesis of T. rangeli into the vector.
Supported by: FAPERJ, CAPES, CNPq

VE014 - **INSIGHT INTO THE SIALOME OF *DIPETALOGASTER MAXIMA*, A VECTOR OF CHAGAS DISEASE, REVEALS THE PRESENCE OF OLIGOMERIC APYRASES**

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Dipetalogaster maxima (Hemiptera: Reduviidae), the largest triatomine known, inhabits sylvatic areas in Mexico. Although *D. maxima* is typically from sylvatic environments where it feeds on lizards, its adaptation to humans has been documented in La Paz, the capital city of Baja California Sur. Many populations of this blood-sucking bug are naturally infected with *Trypanosoma cruzi*. Hematophagous insect salivary glands produce potent pharmacological compounds that counteract host hemostasis, including anti-clotting, antiplatelet, and vasodilatory molecules. Apyrases are ATP-diphosphohydrolases that catalyze hydrolysis of both ATP and ADP to AMP, facilitating blood feeding by degradation of ADP, a mediator of platelet aggregation and inflammation, and ATP, a mediator of neutrophil activation. Partial coding sequences (truncated in the 5′ region) for members of the 5′ nucleotidase family were found in the sialotranscriptome of *D. maxima*. The contig Dm-68 has an extension characteristic of mucins, rich in serine and threonine, when compared with other apyrases/5′-nucleotidases, suggesting that this protein can be highly glycosylated. Proteomic characterization of salivary proteins, using two-dimensional gel electrophoresis and peptide mass fingerprinting, identifies apyrase isoforms from the unique contig Dm-68 in the vicinal spots 1, 2, 3, and 4, each having between three and five ion matches. Furthermore, molecular and enzymographic analysis revealed that apyrases of this insect belong to the 5' nucleotidase family and are oligomeric enzymes. The research on salivary compounds of hematophagous insects stimulates the development of new drugs to be used in disorders of circulatory origin.
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VE015 - **ANALYSIS OF EXPRESSION OF CARBOHYDRATE SITES FOUND ON THE SURFACE OF INTESTINAL EPITHELIUM LUTZOMYIA LONGIPALPIS (DIPTERA, PISYCODIDAE) OF THE STATE OF PARA.**

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American visceral leishmaniasis (AVL) is an infectious disease transmitted by phlebotomine sand flies which Lutzomyia (Lutzomyia) longipalpis is the main species in Brazil. The etiological agent of AVL is a flagellate protozoa of genus Leishmania. The interaction between Leishmania and permissive sand flies is mediated by molecular alterations on Lipophosphoglycan (LPG) side chain present on the Leishmania surface and by expressions of glycoproteins of epithelial insect midgut containing N-acetyl- galactosamine (GalNAc) which probably works as recognition sites by molecules lectin-like present on the Leishmania surface. The aim of this work was to verify the midgut expression of glycoprotein containing GalNAc in specimens of L. longipalpis from Instituto Evandro Chagas-PA colony and from Baracena-PA municipality forest which is an endemic area of AVL. Phlebotomines were dissected in paraformaldeide 4% and each midgut was longitudinally sectioned then incubated with FITC conjugated Helix pomatia lectin (HPA-FITC) that is specific for GalNAc. Lysates of ten midguts of phlebotomine from colony were analysed by SDS-PAGE followed by western blotting with HPA to detect glycoprotein containing GalNAc. Both colony and forest samples showed positive reactivity with HPA-FITC suggesting presence of GalNAc. By use of SDS-PAGE followed by western blotting, the study showed that midguts lysates were GalNAc-positive for displaying peptides ranging from 35 to 75 kDa. The results indicated that glycoprotein containing GalNAc occurs in both phlebotomine from colony and in insects from endemic areas of Barcarena municipality.
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