## VE.01 - BIOCHEMICAL AND ULTRASTRUCTURAL STUDY OF THE INTERACTION PROCESS OF *RHODNIUS PROLIXUS* WITH *TRYPANOSOMA RANGELI*

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*Trypanosoma rangeli* is the second frequently found species of trypanosomes infecting humans in countries of Latin America. Although pathogenic to the vector, to date there is no evidence of pathogenicity of this protozoan to humans. Several molecules have been identified at the surface of these cells. Surface molecules that cleave sialic acid and carbohydrate mapped with the use of lectins are well described for trypanosomatid. However, little is known about the involvement of the release of these molecules by the parasite in the process of interaction with the vector. Data obtained *in vivo* and observed under transmission electron microscope showed that during infection by *T. rangeli* the perimicrovillar membranes exposed on the surface of epithelial cells of the posterior midgut of *Rhodnius prolixus* undergo agglutination. As a result of this assemblage process, the extracellular membranes form a network in some areas of the intestine leaving others unprotected. We believe that these unprotected areas of the intestine epithelium facilitates the penetration of the parasite. The formed network, because of its perfect organization, suggests that a lectin-like molecule may be released by the parasite. Biochemical analysis of conditioned culture medium after epimastigote growth may identify proteins released by *T. rangeli* that induces this perimicrovillar membrane network.
Supported by: UENF, FAPERJ, CNPq / INCT, CAPES.

## VE.02 - *IN VIVO* ANALYSIS OF *Trypanosoma cruzi* AND *Trypanosoma rangeli* DISTRIBUTION OVER TIME IN THE MIDGUT OF *Rhodnius prolixus*

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During the transmission of trypanosome from mammal to vector, midgut is the first place where parasites remain. In this microenvironment, trypomastigote forms differentiate to epimastigotes, the replicative forms in the insect. Although many studies have been developed about *R. prolixus*-trypanosome interaction, the establishment of the infection in the triatomine gut is poorly understood yet. In this study, the distribution of *T. cruzi* (CL strain) and *T. rangeli* (CHOACHI strain) in the *R. prolixus* midgut was evaluated over a period of five days. Fifth instar nymphs were divided into eight groups of five to ten insects. Four groups were infected with *T. cruzi* and four with *T. rangeli*. For this, insects were allowed to feed for one minute in an artificial feeder containing citrated rabbit blood, previously inactivated, with $1x10^7$ parasites/mL. Insects were weighted before and immediately after feeding in order to estimate the number of ingested parasites. Bugs were dissected on 0.5, 24, 48 and 120 hours post-infection and parasite concentrations inside midgut were determined with a Neubauer chamber. For *T. cruzi*, the percentages of recovered parasites were 38, 29, 18 and 0.7 on 0.5, 24, 48 and 120 hours, respectively. For *T. rangeli*, those percentages were 34, 75, 62 and 15. The concentration of *T. cruzi* epimastigotes in the insect midgut diminished over time. For *T. rangeli*, epimastigotes concentrations increased 24h after infection, decreasing only subsequent to this period. In addition, the concentrations of *T. rangeli* inside *R. prolixus* midgut remained higher than those of *T. cruzi* during the period of evaluation. These results suggest different mechanisms of establishment for *T. cruzi* and *T. rangeli* that could have implications on the parasite growth in co-infections. In the next step we will evaluate molecular aspects of those host-parasite interactions in single and mixed infections to confirm such hypothesis. Supported by FAPEMIG and INCT-EM.

## VE.03 - TEMPERATURE AFFECTS THE INFECTION OF *Rhodnius prolixus* BY *Trypanosoma cruzi*

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Environmental temperature and behavioural thermoregulation can determine the course of an infection in several parasite-insect interactions, affecting both parasite virulence and host development. In this study, the effects of temperature on moulting time and mortality of *Rhodnius prolixus* infected by *Trypanosoma cruzi* were evaluated. For infection, second instar nymphs were fed on blood containing cultured epimastigotes of *T. cruzi* CL strain (1 x $10^7$ par/ml). Control insects were fed only on blood. Immediately after the infective meal, insects were transferred to Petri dishes containing filter paper as a substrate and the plates were maintained in temperature-controlled boxes until moulting. After this, insects remained in boxes until completing 90 days of fasting. Temperatures tested were: 21, 24, 27 and 31 °C. The period required to reach third instar varied with temperature and infection status, such that higher temperatures decreased times to moult. Interesting, the infection by *T. cruzi* decreased moulting time in insects exposed to 21, 24 and 27 °C. The infection associated with higher temperatures (27 and 31°C) increased mortality rates, reaching 21 and 96% in infected insects exposed to 27 and 31 °C, respectively (control insects showed 6 and 73% for 27 and 31°C). Moulting rates were not affected by infection or temperature. Results show that the course of infection by *T. cruzi* is affected by environmental temperature, with implications on ecology and evolution of this interaction.
Supported by: CNPq, Fapemig, CPqRR.

## VE.04 - *TRYPANOSOMA RANGELI*: ECTO- PHOSPHOTYROSINE PHOSPHATASE ACTIVITY IS INVOLVED TO *RHODNIUS PROLIXUS* SALIVARY GLAND INTERACTION

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Membrane-bound ecto-phosphatases have been reported as pathogenic factors in various infectious microorganisms and have been characterized in several members of the Trypanosomatidae family. However, the physiological role of these ecto-phosphatases in trypanosomatids had not been well established yet, although they are supposed to be involved in nutrition, protection, virulence and cellular differentiation. In this context we investigated the role of ecto-phosphatase activity of *T. rangeli* during interaction between these parasites and *R. prolixus* salivary glands. In brief, salivary glands of *R. prolixus* were incubated in the presence of long epimastigote form of *T. rangeli* ($10^6$ parasites in 200 μL). Adhesion assays showed a mean of 2000 ± 380 protozoa /pair salivary glands. We examined the capacity of different *T. rangeli* phosphatases inhibitors to modify the protozoa–salivary gland interaction. Addition of sodium orthovanadate, molybdate and $ZnCl_2$ (PTPase inhibitors) significantly inhibited *T. rangeli* adhesion. However, the addition levamisole and sodium fluoride, that not inhibited PTPase activity, did not affect adhesion to salivary glands. To confirm the involvement of PTPase in the binding of protozoa to the salivary gland we add substrates for this enzyme, *p*-nitrophenyl phosphate and phosphotyrosine on *T. rangeli* adhesion. These PTPases substrates inhibited *T. rangeli* adhesion to salivary glands, inhibition that was not observed with others phosphatases substrates, phosphoserine, phosphothreonine and β-glycerophosphate. These results suggest that an ecto-phosphotyrosine phosphatase activity could be involved in interaction *T. rangeli/* salivary gland.
Supported by CNPq, CAPES and Faperj.

## VE.05 - ULTRASTRUCTURAL STUDY OF THE PRODUCTION OF MUCUS AND RECTAL LINING OF *RHODNIUS PROLIXUS*

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The triatomine *Rhodnius prolixus* has been an excellent biological model to study the development of *Trypanosoma cruzi*, the etiologic agent of Chagas disease. In an attempt to block the development of this parasite in the vector, several drugs have been used, among them, azadirachtin has proved to be effective in blocking infection and its use is associated with cellular changes in the intestinal tract. For the evaluation of mucus secretion by intestinal epithelial cells and deposition of polysaccharides in the wax layer of the rectum, ruthenium red was used. The material was processed for routine transmission electron microscopy. Thin sections were observed by bright field microscopy and transmission electron microscopy. Preliminary data obtained by bright field microscopy showed the presence of large amounts of mucus at the stomach of insect control processed with ruthenium red. In insects treated with azadirachtin mucus was not observed. Insects treated with ecdysone and those receiving azadirachtin and ecdysone showed mucus in the lumen similar to insect control. . In the insect control, ultrathin sections stained with toluidine blue, revealed projections lining the lumen of the organ. These projections presented a colored outer line, a pale median line and a colored inner portion with dark lines elongated fibers. Insects treated with azadirachtin presented an increase in the pale median line. However, when administered azadirachtin/ecdysone, the morphological changes caused in wax were not prevented and suggests that different perimicrovillar membranes of the rectum wax can not be regenerated.
Supported by: UENF, FAPERJ, CNPq/INCT, CAPES

## VE.06 - THE INFECTION BY *Trypanosoma rangeli* CHANGES THE SHELTER USE BEHAVIOUR OF *Rhodnius prolixus*

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Triatomines display a daily rhythm of activity with two peaks, a first one corresponding to the search of food at dusk, while the other precedes dawn, and is directed to the search of refuges. We evaluated whether this behaviour is altered in *R.prolixus* after infection by *T.rangeli*. Fourth instar nymphs were infected intracoelomatically with 100 epimastigotes (CHOACHI strain) in PBS, or with PBS only (control). Fifty fifth instar nymphs starved for 30 days were used for each assay (n=4). Assays were conducted in square glass arenas (40x40x20cm) presenting one central refuge of 10 $cm^2$ with two accesses. Assays were done at 24+2°C and a 12:12 DL. The groups of insects were released in the arenas and after 3 days (time allowed for insect acclimatization) any bugs remaining outside shelters were removed. Afterwards, a mouse kept in a container was placed inside the arena to present chemical and vibratory stimuli signaling the presence of a host. Nevertheless, this did not allow the insects to feed. The hosts were presented two hours before starting the scotophase and kept there for 16 hours. The percentage of insects that entered shelters after acclimatization was 67 and 85% for infected and control groups, respectively. Insects from both groups showed an intense activity in the presence of the hosts, leaving and entering the shelters throughout the assays. At the end of these observations, 40% of the infected insects remained outside the shelters, while only 13% of the insects from control group were found in the open arena. These results show that the infection by *T.rangeli* alters the characteristic refuge behaviour of these triatomines, inducing them to become exposed. This may increase triatomine predation by vertebrates and, in the case of mammals, maintain higher parasite transmission rates by predation in nature. Supported by CNPq, Fapemig, CPqRR, FIOCRUZ.

## VE.07 - *Triatoma infestans* SALIVA AS AN ENHANCER OF *T.cruzi* INFECTION.

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*Triatoma infestans (T. infestans),* is a blood-sucking bug from subfamily Triatominea. It is widespread in the Southern Cone countries of South America and it is a vector of Chagas' disease. *Trypanosoma cruzi*, the etiological agent of Chagas' disease, is transmitted by *T. infestans* and while in the triatomine midgut the parasite differentiates from a non-infective epimastigote stage into the pathogenic trypomastigote metacyclic form. An adult *Triatoma* usually ingests two to three times its own weight of blood in a single meal. Blood-sucking insects possess a variety of anti-hemostatic factors in their salivary glands which maintain blood fluidity during feeding. In this work we shows the influence of *T. infestans* saliva in the *T. cruzi* blood parasitemia *in vivo*.The BALB/c mice were separated in two groups of ten animals,the first group received a subcutaneous injection of sterile PBS and the second group received a subcutaneous injection of *T. infestans* saliva. After 5 minutes both groups received a subcutaneous injection of *T. cruzi* (clone Dm28c) with $5\times10^5$ in 100 µl of saline. Blood parasitemia was measured once a week after the seventh day post infection during the following four weeks. The blood was obtained from a small cut at the end of the tail and diluted fivefold in red blood cell lysis buffer and parasite count was measured in a Neubauer chamber. Our results show that the presence of *T. infestans* saliva increase the infection with *T. cruzi* in BALB/c mice. The effect of bug saliva on *T. cruzi* transmission is under study in our lab and we are searching for the eventual role of biomolecules presents in saliva that may enhance parasite transmission. Supported by CNPq, Faperj, IFS

## VE.08 - HEALTH AND ECOLOGICAL ASPECTS OF CHAGAS DISEASE IN THE DISTRICT BNH OF THE CITY OF BARRA DO GARÇAS, MATO GROSSO

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The presence of vectors triatomines *Triatoma williami* homes BNH district in the municipality of Barra do Garças situated near the Serra Azul, has motivated the application of a questionnaire as part of an exploratory research for better understanding of ecological and health aspects of Chagas disease among the inhabitants of the district, thereby identifying basic knowledge on trypanosomiasis and its vectors. Evaluated-some aspects of Chagas disease in urban population of the district BNH, aiming to address the level of knowledge of their residents on trypanosomiasis and characterization of its vectors, in the municipality of Barra do Garças-MT. All the interviews were carried out after accepted consent free. The data contained in the questionnaire were analyzed, used-if the program ArcView GIS version 3.2 in the construction of maps, while data global positioning were measured with GPS Garmin 60 CSx The analyzes obtained show a possible potential of recrudescence of Chagas disease, even with the certificate of interruption of transmission vectorial and, also a potential vector sinantropic *T. williami*. Front of the actual health in Brazil, the scientific community must propose to public bodies of control epidemiological/sanitary greater attention to the combat and control of Chagas disease The process of occupation and disordered the destruction of natural habitat been contributing to drastic environmental changes, increasing the invasion of wild species, besides the existence of diversity of species of triatomines with potential of transmission. There is an enormous challenge to prevent a recrudescence of the transmission of Chagas disease, since one of vectors has presented high degree of synanthropy.
Supported by CAPES.

## VE.09 - MORPHOMETRIC STUDY OF INTRAPOPULATION VARIABILITY OF *TRIATOMA MATOGROSSENSIS* (HEMIPTERA, REDUVIIDAE)

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The insects of the Triatominae subfamily stand out for their importance as vectors of *Trypanosoma cruzi*, the etiologic agent of Chagas disease. The triatomines are distributed throughout the Neotropical regions and the species *Panstrongylus, Rhodnius* and *Triatoma* genus are epidemiologically more important. The genus *Triatoma* is the most numerous and it is subdivided into specific complexes according to morphological similarities and geographic distribution of its species. T. matogrossensis is found in the Central-West region of Brazil and belongs to the oliverai subcomplex, together with *T. baratai, T. guazu, T. jurberg, T. vandae* and *T. williami*. The aim of this study was to determine the *T. matogrossensis* female intrapopulation morphometric variability. For this, we used specimens kept since 02/09/1993 colony in the Insetário de Triatominae, of FCF - UNESP / Araraquara. The original colony was divided according to differences in the size of insects, visually perceptible. For each colony formed, 15 specimens were analysed for parameters of the head (eye diameter, distance ante-ocular and post-ocular and inter-ocular, length of each of the three rostral segments, and head length) and body (body length and length and width of the abdomen and abdomen). According to the results, using unpaired T test, morphometric differences were highly significant for the length of the body, abdomen, head; very significant for length and width of the abdomen, abdomen width, eye diameter and head length; significant for distances ante-ocular and inter-ocular and the second segment; and no significant for post-ocular distance and the length of the first and third rostral segments. We conclude that the parameters used showed significant differences, and there is a real variation in size among the specimens studied, even between individuals of the same species and origin, which justifies the continuity of the observations by other techniques. Supported by: CNPq and Fapesp (Proc. 2010/50355-1)

## VE.10 - PHYLOGENETIC RELATIONSHIP *TRIATOMA* SPECIES (HEMIPTERA, REDUVIIDADE) FROM CENTRAL WEST REGION OF BRAZIL BASED ON CYTOCHROME B GENE SEQUENCE

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The triatomines are vectors of the etiologic agent of Chagas' disease, *Trypanosoma cruzi*. The *Triatoma* genus is the largest and was divided in specific complexes according to morphological similarities and geographical distribution. The seven species studied can be found in the Central West region of Brazil, of which five belong to the oliverai subcomplex (T. *baratai, T. guazu, T. matogrossensis, T. vandae* and *T. williami*) and the other two species, *T. costalimai* and *T. sordida* belong respectively to *T. infestans* and *T. sordida* complexes. The aim of this study was to determine the phylogenetic position of these two species, by comparing the cytochrome b (Cytb) gene fragment sequences of the mitochondrial DNA. The specimens evaluated came from colonies maintained at the Insectary of Triatominae, Faculdade de Ciências Farmacêuticas / UNESP - Araraquara. After extraction of genomic DNA and amplification of Cytb gene fragment, it was sequenced in an automatic DNA sequencer, model ABI 377. The sequences obtained and other sequences (of the same fragment) already available in GenBank were aligned using the Clustal W program, of BioEdit, and the phylogenetic inferences were conducted using the analysis of distance with the MEGA 3.1 program. Sequences of the species *T. sherlocki, T. infestans* and *T. brasiliensis* were included in the analysis to support the phylogeny. The species were distributed in two clades: the first compounded by *T. costalimai, T. sordida, T. matogrossensis* and *T. williami*; and the second compounded by *T. vandae, T. baratai* and *T. guazu*. The phylogenetic analysis using the Cytb sequence show the division of the oliverai subcomplex in two groups and *T. baratai* and *T. vandae* species were included together in one of them. This fragment showed a high degree of polymorphism and homoplasy along the analyzed sequences.
Supported by CNPq and Fapesp

## VE.11 - CHARACTERIZATION OF THE HEMAGGLUTINANT ACTIVITY EXPRESSED IN THE ANTERIOR MIDGUT OF *TRIATOMA INFESTANS* (HEMIPTERA, REDUVIIDAE)

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Hemagglutinins have been described in several hematophagous arthropods. Most of them are lectins with binding specificity for saccharides or lipopolysaccharides. In triatomines, hemagglutination activity was described for different tissues of *Triatoma infestans* and *Rhodnius prolixus*, including haemolymph, salivary glands, anterior and posterior midguts. Each tissue possesses distinct molecules that differ in their binding capacity and their biological role is not known yet. In this study we evaluated the hemagglutinant activity present in the anterior midgut of third instar nymphs of *T. infestans* in different days after molt and partially characterized the hemagglutinin molecule. The insect age after molt influenced significantly the hemagglutinant activity, which is low or absent until approximately 5 days after molt, increases from day 5 to 8 and remains at higher levels from day 7 to more than 25 days. The molecule is thermo-stable and the treatment at 98 °C for 5 minutes doesn't lead to the loss of activity. Hemagglutination was inhibited when midgut extracts were incubated with a nonspecific protease (proteinase K), suggesting that the molecule has a proteic origin. When the midgut extracts were ultra-filtered with 100 kDa cut off membranes, the molecule with hemagglutination activity was present at the portion with molecular weight higher than 100 kDa. New experiments are underway with the aim of identifying the sequence of the molecule, its expression profile and its biological role.
Supported by: FAPEMIG, CAPES and CNPq.

## VE.12 - INFLUENCE OF THE INTESTINAL ANTICOAGULANT IN THE FEEDING PERFORMANCE OF THE TRIATOMINE BUGS (HEMIPTERA; REDUVIIDAE)

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Triatomines are hematophagous insects in all life stages. They are vectors of *Trypanosoma cruzi*, the causative agent of Chagas disease. The vectorial capacity of these insects is directly related to their feeding efficiency, which varies greatly among species due to factors related to the physiology of the host and characteristics of the insect feeding apparatus. Only recently, factors found in the gut environment were reported as influencing the feeding process, as hemagglutination and blood clotting. In this work, we investigated the level of anticoagulant activity achieved by the intestinal contents of three species of triatomines - *Triatoma infestans, Triatoma brasiliensis* and *Rhodnius prolixus* - and correlated the anticoagulant activity of each species with their feeding efficiency on live hosts. For all studied species, the anticoagulant activity was significantly higher in the anterior midgut (crop) contents than in saliva. Among the species, *T. brasiliensis* had the lowest crop anticoagulant activity, the lowest concentration of thrombin inhibitor, and is also the specie that was verified higher difficulty in the feeding process. To confirm the findings that the anticoagulant activity magnitude interferes with the blood pumping into the crop, we knocked down by RNAi the expression of brasiliensin, the intestinal thrombin inhibitor from *T. brasiliensis.* The brasiliensin knockdown nymphs had lower capacity to maintain the cibarial pump contractions frequency throughout the feeding process even in favorable conditions (feeding on a large diameter vessel), and lower blood ingestion rate (mg/min), when compared to control nymphs. However, the difficulty during feeding was reversed in brasiliensin knockdown nymphs fed on mice treated with heparin (a potent systemic anticoagulant), that behaved similarly to the control nymphs. Thus, the intestinal anticoagulant activity is directly related to the blood-pumping frequency modulation, which affects the feeding performance of triatomine bugs.
Supported by: FAPEMIG, CAPES and CNPq.

## VE.13 - THE KNOCKDOWN OF CATALASE AND DUAL OXIDASE INTERFERES WITH OVIPOSITION AND ECLOSION RATES IN *RHODNIUS PROLIXUS*

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The regulation of the generation and elimination of reactive oxygen species (ROS) is key to aerobic life. Here, in order to evaluate the role of dual oxidase (Duox, an $H_2O_2$ producer) and catalase (Cat, an $H_2O_2$ scavenging enzyme) in *Rhodnius prolixus*, we used RNAi to knockdown expression of Duox and Cat genes by injection of gene-specific double-stranded (ds) RNAs into the haemocele of female insects. qPCR showed inhibition of the expression of both genes after the blood meal, reaching 99,5 and 99,7% for Cat and Duox, respectively. In the insects injected with Cat dsRNA, the oviposition and eclosion rates were, respectively, 50 and 75% smaller in comparison with control insects. In the insects injected with Duox dsRNA the oviposition rate was not altered. However, eclosion of first instar was dramatically reduced in these insects, from 55% to 100 % of the control insects. Together, these results show that control of redox metabolism is essential both to oogenesis and embryogenesis.
Supported by CNPq, CAPES, HHMI and FAPERJ.

## VE.14 – Insight into the salivary transcriptome and proteome of *Dipetalogaster maxima*

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*Dipetalogaster maxima* is a blood sucking hemiptera that inhabits sylvatic areas in Mexico. It usually takes his blood meal from lizards but the human population growth resulted in bugs invading suburban areas, feeding also in humans and domestic animals. Hematophagous insects' salivary glands produce potent pharmacological compounds that counteract host hemostasis, including anti-clotting, anti-platelet, and vasodilatory molecules. To obtain a further insight into the salivary biochemical and pharmacological complexity of this insect, a cDNA library from its salivary glands was randomly sequenced. Also, salivary proteins were submitted to one and two dimensional gel (2D-gel) electrophoresis followed by MS analysis. We present the analysis of a set of 2,728 (SG) cDNA sequences, 1,375 of which coded for proteins of a putative secretory nature. Most salivary proteins were described as lipocalins, corresponding to 93% of the transcripts coding for putative secreted proteins. Lipocalins are a large and heterogenous group of proteins that play various roles, mainly as carriers of small ligands in vertebrates and invertebrates. A great array of salivary gland proteins belonging to the lipocalin family has generated a large number of different molecules having anti-hemostatic functions while maintaining the fundamental structure of the protein fold. Lipocalins were found in the saliva of other blood-sucking triatominae bugs as *Rhodnius prolixus*, *Triatoma brasiliensis*, *Triatoma infestans*, and also in tick saliva.
Supported by: CNPq, Finep, FAP-DF, NIH

## VE.15 - INTERACTION OF *PHYTOMONAS SERPENS* WITH THE PHYTOPHAGOUS INSECT *ONCOPELTUS FASCIATUS* (MILKWEED BUG)

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The hemipteran insect *Oncopeltus fasciatus* (milkweed bug) is found in several U.S. states, Mexico and Brazil. Due to its high reproductive activity in the laboratory, short life cycle and size large enough to be easily handled, this insect is used as a model for various studies, including host-parasite interactions, both in natural and experimental conditions. *O. fasciatus*is the natural host of a number of species of the genera *Phytomonas, Crithidia* and *Leptomonas*. Our laboratory colony of *O. fasciatus* is naturally infected with *Leptomonas wallacei*. We have obtained protozoan-free *O. fasciatus* by treating the eggs with sodium hypochlorite. After eclosion, the insects were fed with peeled sunflower seed and mineral water. The absence of trypanosomatids in the gut was confirmed by both scanning electron microscopy and PCR, using primers specific for *L. wallacei*. A colony of *O. fasciatus* free of trypanosomatids has been kept apart from the original colony. Plant trypanosomatids *Phytomonas* have recently attracted attention due to their role as agricultural parasites of both plants and insects. Little is known of the life cycle of *Phytomonas* species in the insect hosts, despite its paramount importance for the transmission of these flagellates to their plant hosts. While *Leptomonas* spp colonize only the digestive tract of their hosts, *Phytomonas* spp cross the intestinal epithelium, reach the hemolymph and infect the salivary glands. Trypanosomatid-free insects were used for assays of interaction with *P. serpens*. Either these parasites or vehicle were injected with a microsyringe into the thorax of adult *O. fasciatus* by puncturing the articulation of a prothoracic coax. At different times from 6 to 72 h after the injections, the insects were lightly anesthetized on ice; the hemolymph was collected from 8 to 10 insects per treatment group, by cutting off metathoracic legs and gently pressing the abdomen. Large numbers of parasites were observed in Giemsa-stained smears from the hemolymph, and interaction of *P. serpens* with hemocytes was examined by both light and transmission electron microscopy. The insects were carefully dissected and their intact salivary glands extracted, fixed and examined by means of scanning electron microscopy. Parasites were found attached to a dense extracellular layer, the basal lamina, closely associated through both the flagellum and the cell body of *P. serpens*. On the other hand, invasion of the basal lamina occurred only via the protozoan cell body. Parasites were also found under the basal lamina, attached to the outer surface of the salivary gland, as well as entering the lumen of the gland. *P. serpens* promastigotes were also allowed to interact with extracted salivary glands from *O. fasciatus*. The basal lamina of the salivary glands showed holes suggestive of parasite penetration. SDS-PAGE-gelatin gels prepared with supernatants of the medium of interaction showed that both the parasites and the salivary glands produce proteinases. The parasites release a 63 kDa enzyme consistent with a metallo-proteinase; proteins released from the salivary glands also showed a proteinase profile consistent with a metallo-proteinase (15 kDa).

Supported by: CNPq, FAPERJ, CAPES, PIBIC-UFRJ and INCT-EM.

## VE.16 - PHLEBOTOMINE FAUNA (DÍPTERA: PSYCHODIDAE) DISTRIBUTION ACROSS AN URBAN-RURAL GRADIENT OF A VISCERAL LEISHMANIASIS ENDEMIC AREA IN THE MUNICIPALITY OF BARCARENA, PARA, BRAZIL.

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American visceral leishmaniasis (AVL) is a zoonosis caused by a protozoan of specie *Leishmania (Leishmania) infantum chagasi* and it is transmitted by phlebotomines sand flies of genus *Lutzomyia*. The disease was typically rural, but it has become urbanized in consequence of drastic environmental alterations caused by human action. Nowadays AVL has been verified in many Brazilian cities including Santarem (PA), Fortaleza (CE), Rio de Janeiro (RJ) and Belo Horizonte (MG). The aim of this study was to verify the urbanization of *Lutzomyia (Lutzomyia) longipalpis,* vector of AVL in the municipality of Barcarena-PA, Brazil. Systematic captures of phlebotomines were performed using CDC light traps. The captures were carried out in areas of forest, edge of forest, intermediate area and urban area, from 2007 to 2009. A total of 5,089 specimens belonging to eleven species were collected, with predominance of *L. (L.) longipalpis* (95.15%), *Lutzomyia (Sciopemyia) sordellii* (2.06%) and *Lutzomyia (Nyssomyia) flaviscutellata* (1.76%). The highest population densities (88.25%) were from edge of forest belonging to a locality occupied about twelve years. However, the visceral leishmaniasis vector was not captured in urban area, suggesting it has not been urbanized in Barcarena yet. Another two species, *Lutzomyia* (*Nyssomyia*) *flaviscutellata* and *Lutzomyia (Psychodopigus) paraensis* related with cutaneous leishmaniasis transmissions were captured in the forest and edge of forest. More than seventy percent of all *L. (L.) longipalpis* specimens captured were males, whereas to another species, the number of females was more frequent than males. For the diagnosis of infection rate, the microscope analysis was performed but all analyzed samples were negative. These results suggest low rate infection in these areas. The presence of phlebotomine specimens captured in edge of forest reinforces the necessity of entomological monitoring in Barcarena municipality. Supported by CNPq and UFPA.

## VE.17 - CASPAR AND TGF-BETA ARE POTENTIALLY INVOLVED IN *LUTZOMYIA LONGIPALPIS*-PATHOGEN INTERACTION

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*Lutzomyia longipalpis* is the main vector of visceral leishmaniasis in Brazil. In order to identify important events in vector-pathogen interaction we have sequenced ESTs of cDNA libraries from *L. longipalpis* gut RNAs obtained after blood feeding, and after infection with *Leishmania*. Among the sequences we have identified a cDNA coding for a TGF-beta closely related to the Activin subfamily, which regulates immune response and wound repair among other functions. The transcriptional profile of this gene was studied by semi-quantitative RT-PCR using RNA extracted at different times after blood feeding or artificial infection with *Leishmania infantum chagasi*. TGF-beta transcription increased 72h after infection when compared to control uninfected samples. Interestingly, this is the time when the perithrophic matrix degrades and the parasites attach to the vector midgut. Western blot experiments are being performed to investigate the kinetics of protein production. We are also interested in investigating the involvement of the different innate immune pathways in the response to pathogens. We identified a sequence similar to Caspar, a repressor molecule of the IMD pathway. The transcriptional profile of Caspar was determined by semi-quantitative RT-PCR using RNA extracted at different times after blood feeding or artificial infection with *Leishmania mexicana.* Caspar transcription decreased after 72h infection when compared to blood fed samples. RNAi experiments were performed to access the gene function. Females were microinjected with Caspar dsRNA and 72h later were infected with *L. mexicana*. Caspar knocked-down insects showed reduction of parasite count in the midgut when compared to control groups. This phenotype indicates that the non-repressed IMD pathway is capable of reducing *Leishmania* survival in the insect midgut. This is the first report of an immune related gene in sand flies affecting *Leishmania* survival. Supported by: CAPES, CNPq, FAPERJ, Fiocruz.

## VE.18 - PHYLOGENETIC RELATIONSHIPS AMONG FOUR SPECIES OF RUBROVARIA SUBCOMPLEX USING DNA SEQUENCE OF THE MITOCHONDRIAL CYTOCHROME B GENE

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Currently 140 species of Triatominae are recognized. In recent studies, the genus *Triatoma* was grouped into eight complexes and subcomplexes. *Triatoma rubrovaria*, *T. circummaculata*, *T. klugi* and *T. carcavalloi* species are found in similar ecotopes in the Rio Grande do Sul State, Brazil, and they are grouped into one subcomplex (rubrovaria subcomplex), based on morphological characteristics. Aiming to verify the relationship of genetic similarity between those species, the cytochrome B (cytB) fragments were sequenced in this four species. The consensus sequences were evaluated for phylogenetic relationship using the distance methods by the Neighbor Joining algorithm, under the Kimura 2-parameter model implemented in MEGA 4.0 software. The support for the groups was evaluated using distance bootstrap analyses with 500 replicates. The phylogenetic tree was rooted using the cytB sequence of *T. dimidiata*. The phylogenetic analyses using the cytB sequences showed a close relationship between *T. klugi* and *T. carcavalloi* and between *T. rubrovaria* and *T. circummaculata*, agreeing with the morphological data and the current rating.
Supported by FAPESP, 2009/52236-2

## VE.19 - EVALUATION OF *AEDES AEGYPTI* COLONIZATION BY *BLASTOCRITHIDIA CULICIS*

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In a previous work, we demonstrated that *Blastocrithidia culicis,* an endosymbiotic-harbor monoxenic trypanosomatid, is capable of colonizing the mosquito's digestive tract and crossing the intestinal epithelia, reaching the haemocoel of artificially fed *Aedes aegypti*. In order to further characterize this interaction we analyzed midgut and salivary gland proteins that are able to recognize *B. culicis.* With this purpose, mosquito midgut and salivary gland proteins were analyzed by blotting assays using *B. culicis*-biotinylated-epimastigotes as a ligand. In salivary glands we observed five proteins that recognize *B. culicis,* of which two were identified by Edman degradation as apyrase and aegyptin. In the midgut, biotinylated-epimastigotes bound to seven proteins with molecular mass ranging from 10 -50 KDa. All these mosquito midgut proteins are glycoproteins, as they were labeled by different lectins. We also demonstrated that radiolabeled-protozoa inoculated into the mosquito thorax bound to midgut, salivary glands and ovaries. Here, we analyzed the *B. culicis* - *A. aegypti* colonization after feeding mosquitoes with epimastigotes, and following the presence of the protozoa in different organs during 48 days using polymerase chain reaction (PCR). *B. culicis* was detected in midgut, hindgut, abdomen, crop, Malpighian tubules and ovaries early after feeding. Protozoa were detected in salivary glands 28 days post-feeding. These data confirmed our previous assay using radiolabeled-epimastigotes. Taken together these results evidence aspects of *B. culicis* life cycle in *A. aegypti*.
Support: CAPES, CNPq and FAPERJ.

## VE.20 - INITIAL CHARACTERIZATION OF *CULEX QUINQUEFASCIATUS* VITELLOGENIN RECEPTOR

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As occurs with haematophagous insects, *Culex quinquefasciatus* vitellogenesis is triggered by a blood meal. Besides the synthesis of yolk proteins by the fat body, the process is characterized by the development of the endocytic complex at the apical surface of the oocyte, which will allow the uptake of the large amounts of nutrients needed for embryonic development. The endocytic complex is composed of microvilli, specific receptors, mainly vitellogenin receptor (VgR), clathrin-coated vesicles and endosomes. The *Cx. Quinquefasciatus* vitellogenin receptor (CxVgR) cDNA was sequenced using primers based on the *Aedes aegypti* VgR (L77800), since at this time the molecular database of *Cx*. *pipiens quinquefasciatus* was still incomplete. The sequence of the PCR products presented high similarity to the *Ae. aegypti* (L77800) and *Cx. pipiens quinquefasciatus* (CPIJ020278) sequences and the amino acidic deduced sequence showed typical characteristics of the family of receptor low density lipoprotein receptor (LDLR). This family contains five distinct domains: a ligand-binding domain; epidermal growth factor (EGF)-like repeats; repeats containing a YWTD motif; a transmembrane domain anchoring the receptor to the plasma membrane and a cytoplasmic domain. RT-PCR showed that the transcript is present, only in the ovary samples, already in the first day after emergence (AE) and increases progressively until the fifth day AE. During the gonotrophic cycle, the gene increases until the 48 h post blood meal. These results were confirmed by real-time PCR and correlate with females engorgement behaviour and with their fecundity and fertility.
This work was supported by FAPESP.

## VE.21 - CHARACTERIZATION OF *CULEX QUINQUEFASCIATUS* HEXAMERINS

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Insect fat body is a multifunctional organ involved in storage and intermediary metabolism of lipids, carbohydrates and proteins. The analysis of fat body extracts of juvenile and adult stages of *Culex quinquefasciatus* by one-dimension SDS-PAGE revealed the presence of several major proteins with molecular masses between 74 and 91 kDa in the last instar of both larval and pupal stage. The major proteins of the pupae fat body extract were submitted to an *in gel* reduction, alkylation, and trypsinization and resulting peptides were analyzed by mass spectrometry, revealing that these proteins belong to different groups of insect hexamerins. Insect hexamerins are storage proteins with a native molecular mass of around 500 kDa, consisting of a random association of quantities and types of immunologically related homologous subunits (approximately 70-85 kDa each). These proteins, which are synthesized and secreted into the haemolymph during the last larval stage, are incorporated into granules of the fat body cells in the pre-pupal stage. In some genera, hexamerins can be used during the non-feeding phases or during metamorphosis. Moreover, hexamerins act as an amino acid reserve for protein production, mainly during cuticle development of pupae and adults. We are currently analyzing the gene expression profile of *Cx. quinquefasciatus* hexamerins to determine the sites of synthesis and storage.
Supported by FAPESP. LAM and ACF are FAPESP fellowships.

## VE.22 - ANALYSIS OF THE PROMOTER REGION FROM A *LUTZOMYIA LONGIPALPIS* CHITINASE GENE

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Leishmaniasis are diseases with high epidemiological impact that affect millions of people around the world. Chitinases are important enzymes in insect biology and, since the 1990s, their involvement in the parasite – vector interaction process is being studied. In *Lutzomyia longipalpis*, the main vector for visceral leishmaniasis in America, the chitinolytic activity in the gut of blood fed females seems to be fundamental for modulation of the peritrophic matrix (PM) thickness and may influence the infection of the vector by the parasite. A *L. longipalpis* chitinase cDNA (Llchit) potentially involved in PM degradation was isolated from blood fed females and characterized. Then, a genomic clone, containing the gene coding for this cDNA, was isolated from a genomic library and sequenced, revealing the presence of 4 introns. The 5`flanking region (FR) of the chitinase gene, present in the genomic clone, was also sequenced and submitted to analysis *in silico*, revealing the presence of a possible promoter region. In order to obtain larger flanking regions, genomic DNA of *L. longipalpis* was subjected to reverse PCR and the products were cloned and sequenced. As a result, we obtained a total of 1270bp of the FR5`. Subsequent bioinformatics analysis identified regions responsive to transcription factors E74 and Kr, initially identified in *Drosophila melanogaster*. E74 regulates mosquito genes after a blood meal and also participates in the chitin synthesis pathway. We also cloned the FR5` in pGL3 basic vector (Promega), to investigate its ability to induce the expression of luciferase in *L. longipalpis* cultured cells (LL5), since we showed previously that these cells express Llchit RNA.
Suported by CNPq, FAPERJ, PDTIS.