Vetores - Vectors

VE01 - Real-time PCR quantitative study of Trypanosoma cruzi colonization in Rhodnius prolixus vector

da Silva, RI *(FIOCRUZ)*; Lara, FA *(UFRJ)*; Fragoso, SP *(IBMP)*; Cardoso, MA *(FIOCRUZ)*; Britto, C *(FIOCRUZ)*; Salmon, D *(UFRJ)*

Trypanosoma cruzi epimastigotes are capable of replicating inside the gut of the hematofagic insect vector, afterwards migrating to the rectum where the parasites differentiate into metacyclic tripomastigotes. With the aim of studying the trypanosome colonization process in the vector, an established transgenic lineage of *T. cruzi* Dm28c which expresses the GFP (Green Fluorescent Protein) was used. This approach enables parasite visualization by epifluorescence microscopy inside each compartment of the insect digestive tube. Fifth stage nymphs of *R. prolixus* were artificially fed with blood or decomplemented serum containing cultured epimastigotes (expressing or not the GFP). During six weeks after feeding, at variable intervals, the insects were dissected and the tissues fixed prior to microscopic analysis. In order to establish the relative percentage of parasites in each digestive tube compartment, total DNA was obtained from the respective tissues and submitted to amplification by real-time PCR. DNA satellite conserved sequences from *T. cruzi* were utilized as targets for the design of specific primers and a fluorogenic probe (TaqMan system). Microscopic observation using infected insect nymphs fed with reconstituted blood suggest that the parasite preferentially colonizes the vector stomach (anterior mid gut), forming visible clusters until three weeks after feeding. Parasites were not detected in the insect posterior mid gut one week after the first alimentation. After a second meal, real-time PCR revealed an increment of *T. cruzi* in the rectum, which might be correlated with a massive process of differentiation into metacyclic forms, as observed by epifluorescence microscopy. Microscopic observation of *R. prolixus* fed with infected serum indicated an absence of parasite colonization in all tissues analyzed. Herein we propose to quantify the relative number of parasites present in each tissue, in order to confirm the microscopic analysis and establish the *T. cruzi* colonization kinetics in *R. prolixus* vector after a first meal.

VE02 - Differential genic expression in response to Plasmodium-Mosquitoes Interaction

Araujo, R.V. *(USP)*; Ifa, D.R. *(UNICAMP)*; Souza, G.H.M.F. *(UNICAMP)*; Eberlin, M.N. *(UNICAMP)*; Capurro, M.L *(USP)*

Using Plasmodium gallinaceum infected Aedes aegypti we have analyzed differences in mRNAs profiles that reflect changes in transcriptional activity of genes during infection. We monitored concentration of 9 different transcripts during the first gonotrophic cycle of infected and uninfected mosquitoes by RT-PCR. Ours results showed that P.gallinaceum infection results in decrease on temporal abundance of vitellogenin (Vg) (60%), vitellogenic carboxypeptidase (VgCx) (30%) and vitellogenic cathepsin B (VgCt) (25%) mRNAs, all of them produced by fat bodies. On the other hand, no differences were observed on mRNA levels of ovary transcribed genes, as those coding for Vg-receptor (VgRc) and lipophorin-receptor (LpRc). In addition, the experimental protocol was used to compare mRNA abundance of lipophorin (Lp) and transferrin (Tr), major transport proteins and defensin A (Df) synthesized by fat bodies, and ferritin (Fr) that is synthesized by midgut cells. Our results show that during the first gonotrophic cycle, no differences on profile of Lp, Tr, Df and Fr transcripts were detected between control and infected mosquitoes. Nevertheless, the transcripts levels of Lp, Tr and Df were increased between 7th and 8th days post-infected blood feed. These results suggest that differents groups of genes response to the oocyst development and sporozoites presence on hemolymph. Another approach in our research group is the study of infected Aedes aegypti hemolymph proteins using proteomic methodology. Our preliminary data of 2D eletrophoresis show that in the hemolymph of P. gallinaceum infected mosquitoes 16 spots have no difference on the expression while 6 spots increase and 6 spots decrease in concentration. We also found that 5 spots are expressed only in non-infected mosquitoes and 12 spots are detected only on infected mosquitoes. The mass spectrometry analyses by MALDI-TOF are ongoing and offer a new way to understand host-parasite interaction. Supported by FAPESP and CNPq

VE03 - Cloning and expression of *Rhodnius prolixus* heme binding recombinant protein

Scofield, V. *(UFRJ)*; Lisboa, L.B. *(UFRJ)*; Paiva-Silva, G.O. *(UFRJ)*; Oliveira, P.L. *(UFRJ)*

Rhodnius prolixus, the vector of Chagas disease, an important human sickness, is an hematophagous insect, which ingest large amounts of blood in a single meal. This blood, when digested, release large amounts of free heme in the insect midgut lumen. Free heme generates powerful reactive oxygen species, which damage a lot of biological components of the cells, like lipids, proteins and DNA. Therefore, this insect has developed several mechanisms to deal with this oxidative challenge, which allowed it to adopt blood as source of nutrients. One of these mechanisms is Rhodnius Heme Binding Protein (RHBP). This protein binds one molecule of heme, impairing its prooxidant activity. In this work we isolated RHBP gene from *R. prolixus* female fat body, by RT-PCR. The full-length sequence of RHBP cDNA encodes a pre-protein of 128 amino acids with low similarity with non-annotated proteins from *Drosophila melanogaster* and *Anopheles gambiae*. The first 19 amino acids represent a signal peptide for secretion. RHBP cDNA without the signal peptide was cloned in pDONR 201 vector followed by recombination into the expression vector pDEST 14 using Gateway

cloning system. DH10B and BL21 *E. coli* strains were transformed for stocking and cDNA expression respectively. Finally, we intend to purify and crystallize recombinat RHBP to determine its structure - free and bound to heme - by X-Ray diffraction. Supported by CNPq, FAPERJ, PRONEX, PADCT, HHMI.

VE04 - EST sequencing of Lutzomyia longipalpis cDNA libraries constructed from non-infected and leishmania infected gut.

Beteille V.A. *(Fiocruz)*; Mejido D.C.P. *(Fiocruz)*; Mello T.V. *(Fiocruz)*; Pimentel B.R. *(Fiocruz)*; Ortigao-Farias J.R. *(Fiocruz)*; Moulin-Batista L. *(Fiocruz)*; Lobo A.R. *(Fiocruz)*; Pitaluga A.N. *(Fiocruz)*; Traub-Cseko Y.M. *(Fiocruz)*

Leishmaniasis is a serious public health problem in the whole world. In Brazil, there is a large distribution of the disease, with recent epidemic outbreaks. According to WHO, the endemic regions for leishmaniasis, as well as case numbers, have increased. In Brazil, visceral leishmaniasis is caused by *Leishmania chagasi*, transmitted mostly by *Lutzomyia longipalpis*. Although these insects serve as important vectors for leishmaniasis and other disease, there is little molecular information available. In this aspect, this work aims to identify the transcriptome of *L. longipalpis* gut, infected or not with leishmania, using cDNA libraries constructed in Lambda Zap. Libraries were generated from RNA extracted from *L. longipalpis* gut 72 hours after blood meal and 72 hours after artificially infection with *L. chagasi*. These libraries were excised and so far 606 clones have been sequenced, with 519 sequences showing high quality, adding up to 476 Kb. In the infected library we found 70 singlets and 19 clusters and in the control library 63 singlets and 27 clusters, with little redundancy. Clusterizations are being confirmed and new BlastX searches are being performed after the addition of the remaining EST sequences that are being obtained. Forty seven sequences without any hit after BlastX search against GenBank were identified; these sequences will be re-analyzed using specific programs for the identification of putative new genes. In both libraries, several genes related with sugar metabolism and blood meal digestion were found, such as trypsin, chymotrypsin and ferritin. We expect to have approximately 3000 sequences at the end of the project. This EST sequencing project will be useful in the identification of genes related to *L. longipalpis* metabolism and physiology with special emphasis in blood meal digestion and *L. chagasi* infection.

VE05 - Polymorphism of the ITS-2 region of the ribosomal DNA of *Rhodnius domesticus* (Neiva & Pinto, 1923), *R. pictipes* (Stäl, 1872), *R. prolixus* (Stäl, 1859) and *R. stali* (Lent, Jurberg & Galvão, 1993).

Naegele, M. P. *(UNESP)*; Costa, P. I. *(UNESP)* Rosa, J. A. da *(UNESP)*

Some species of Triatominae bugs are very alike in coloration and in morphological characteristics, specially the ones that belong to the genus *Rhodnius* and for this reason there is some difficulty in identifying them for sure. Many of the techniques of molecular biology have been applied to the identification and systematics of the Triatominae. In the present paper, the species *R.domesticus* and *R. pictipes* have been studied comparatively with *R. prolixus* and *R. stali*, using the polymerase chain reaction-based restriction fragment length polymorphism technique (PCR-RFLP). The genomic DNA of each species was extracted from the legs and the ribosomal ITS-2 region of the DNA was amplified, generating identical bands of 1200 bp. These bands were digested separately with eleven restriction endonucleases and the resulting fragments were separated by electrophoresis in 2.5% agarose gel. Among the enzymes employed, Mbol and Rsal were able to distinguish two groups of *Rhodnius*, putting *R. domesticus* and *R. prolixus* in one group and *R. pictipes* and *R. stali* in another. HaeIII and HinfI showed restriction sites in all four species, but all of them gave the same fingerprinting pattern for the two enzymes. AccI, EcoRI, EcoRV, HindIII and NotI did not show restriction sites in the ITS-2 regions. Three out of the four species were identified by BstUI and HhaI. BstUI was able to differentiate *R. domesticus*, *R. pictipes* and *R. prolixus*, while HhaI differentiated *R. domesticus*, *R. pictipes* and *R. stali*. With the fingerprinting analysis generated by these two enzymes, it is possible to identify very clearly all four species in the study.

VE06 - Proteomics of Triatoma brasiliensis (Reduviidae, Triatominae) salivary gland

Santos, A *(UFMG)*; Lehane, MJ *(LSTM)*; Ribeiro, JMC *(NIH)*; Veloso, AB *(UFMG)*; Araújo, RN *(UFMG)*; Gontijo, NF *(UFMG)*; Pereira, MH *(UFMG)*

Blood feeders produce biomolecules in their saliva to counteract vertebrate host haemostasis events as anticoagulants, vasodilators and inhibitors of platelet aggregation. Besides these agents, other bioactive molecules, which are also probably involved in the feeding process, have been described, such as an antihistamine component, a sialidase, a serine protease, a sodium channel blocker, immunosuppressants and poreforming molecules. Triatoma brasiliensis is the most important Chagas disease vector in the North-eastern of Brazil, colonizing both sylvatic and domestic environments. Despite the epidemiological importance of this species, very little is known about its saliva. In order to investigate the bioactive salivary molecules of the triatomine bug Tri-

atoma brasiliensis, we performed mass sequencing of a salivary gland cDNA library, obtaing 2,600 sequences that were analysed and grouped in 975 contigs. After BlastX analysis we could separate the sequences as follows: 361 (37%) corresponding to probably secreted proteins, 258 (26.5%) are probably housekeeping genes and 356 (36.5%) have unknown function. According to the best match to non-redundant protein database, among the probably secreted proteins, we could observe that most of them were lipocaline-like proteins such as nitrophorin, triabin, pallidipin described for other triatomines. Other interesting genes with similarity to an anti-complement protein, an apyrase, an antigen 5 precursor and a kazal protease inhibitor, were also found. Fifty five contigs were chosen considering their predicted function, E-value and Edman degradation results from salivary purified proteins to perform complete sequencing. The functions of some of these genes will be better analysed using functional genomic techniques, such as RNAi and recombinant protein expression. Supported by: CNPq, FAPEMIG and Wellcome Trust

VE07 - Comparative proteomic analysis of the saliva of *Triatoma infestans*, *Rhodnius prolixus*, *R. brethesi*, and *Panstrongylus megistus* reveals different patterns of protein expression

Sébastien Charneau *(UnB - LBQP)*; Jaime Paba *(UnB - LBQP)*; Carlos A. O. Ricart *(UnB - LBQP)*; Jaime M. Santana *(UnB - LMPDC)*; Antonio R. L. Teixeira *(UnB - LMPDC)*; Marcelo V. de Sousa *(UnB - LBQP)*

Haematophagous triatomine bugs produce pharmacological substances in their saliva to counteract vertebrate host haemostasis events such as coagulation, vasoconstriction and platelet aggregation, and to modulate vertebrate host immune-response. Moreover bugs generate bactericides and anti-fungi compounds in their saliva to preserve their integrity. *Triatoma infestans* and *Panstrongylus megistus* in the Cerrado biome and *Rhodnius brethesi* and *R. robustus* in the Amazonian rain forest are important Chagas disease vectors in Brazil. To investigate the bioactive molecules in the saliva of each of these four bugs, proteomic analysis of salivary gland secretions was initiated. Experimental conditions for two-dimensional electrophoresis (2-DE) analyses were optimized. Comparative analysis of the 2-DE maps of each saliva species revealed different expression profiles among genera. The identification of the spots formed by secreted salivary proteins from the 2-DE maps is currently under progress by MALDI-TOF MS. This first attempt resulted in the identification of the anti-haemostatic "biogenic amine-binding protein", which appears to be major protein in *Rhodnius* saliva. Other approaches such as tandem mass spectrometry MS/MS and Edman chemical sequencing are being used as second alternative strategy for protein identification. New molecules with potential therapeutics of human diseases and control of *Trypanosoma cruzi* transmission could be discovered by the proteomic analyses of the triatomine's saliva. Additionally the comparative analyses of these salivary proteomes will lead us to understanding the features of divergent evolution that have been associated with different triatomine's biological behaviours.

VE08 - Effects of Azadirachtin on the Development of *Trypanosoma rangeli* in *Rhodnius prolixus*

Côrte-Real R *(Fiocruz)*; Castro DP *(Fiocruz)*; De Figueiredo MB *(Fiocruz)*; Garcia ES *(Fiocruz)*; Henriques-Pons A *(Fiocruz)*; Azambuja P *(Fiocruz)*

To investigate the relationship between the neuroendocrine system and the immune defense reactions, the treatment with azadirachtin A and infections with epimastigotes of *Trypanosoma rangeli* strain H14 in 5^th-instar larvae of *Rhodnius prolixus* were performed. The insects were fed on human citrated blood containing 1 x 10^6 epimastigotes/ml and/or 1μg of azadirachtin/ml A. The groups of larvae previously fed on blood alone and blood containing azadirachtin received hemocelic inoculation of 1μl of 1 x 10^7 epimastigotes/ml at 5 days after feeding/treatment. The main results observed were: (i) the number of parasites in the digestive tract significantly increased when combining oral infection and oral treatment with azadirachtin, in comparison to the control group not treated with azadirachtin; (ii) the parasites apparently did not penetrate the intestinal epithelial cells to infect the hemocelic cavity of vector; (iii) there were no effects of azadirachtin on mortality and hemocyte microaggregation in the hemolymph of insects orally infected; (iv) the treatment with azadirachtin was capable of reducing the prophenoloxisase-activation in insect injected with parasites. These results indicate that azadirachtin interfere in the *Trypanosoma rangeli* development in *Rhodnius prolixus* larvae inoculated or infected orally. Supported by: CNPq/Fiocruz

VE09 - Mosquito Phosphotyrosine Profile is Manipulated by Malaria Parasite Infection.

Senna, R. *(UFRJ)*; Silva-Neto, M.A.C. *(UFRJ)*

In recent years malaria research has focused in detailing the molecular events occurring within mosquito upon infection by the parasite. Many events are observed in adult females in response to an infected-blood meal and then require several signaling cascades, most of which are still unknown. The final target of every cell-signaling pathway involves the reversible phosphorylation of proteins. Therefore, the present study was designed to identify the complete set of phosphoproteins whose phosphorylation states are modified by malaria parasite. Firstly, to identify these proteins, *Aedes aegypti* females were blood-fed in *Plasmodium gallinaceum* infected chickens. One and 7 days after blood meal females were blotted against anti-phosphotyrosine. Such strategy determined a set of proteins that change their phosphorylation profile at two different phases of the parasite life cycle within the mosquito. Tyrosine phosphorylation profile was

first evaluated once malaria parasite is devoid of classical tyrosine kinases. Therefore, changes on tyrosine phosphorylation profile are likely to be atributted to the vector set of phosphoproteins. Mosquito females 24 hours after infection were dissected in two segments: head + torax (HT) and abdomen/ovary (AO). HT and AO were homogenized and blotted against anti-phosphotyrosine. We employed tyrosine phosphatase assay as an overall sensor of changes and we observed that infection induced a decrease in midgut tyrosine phosphatase activity. HT and AO samples were then immunoprecipitated with an immobilized-phosphotyrosine antibodies and silver stained. We observed that this second strategy was sensible enough to entrap a set of phosphoproteins present only on infected-mosquitoes. Identification of phosphoproteins is currently being conducted by 2D electrophoresis. In conclusion, the results are sound evidence for the identification of signaling scaffolds required for the parasite to achieve infection and may represent in the future a novel strategy to control malaria transmission by mosquitoes. Supported by: CNPq, FAPERJ, IFS and PADCT.

VE10 - Phytomonas serpens: cysteine peptidase inhibitors interfere with growth, ultrastructure and host adhesion

Camila Guarany *(UFRJ)*; Fernanda Machado *(UFRJ)*; d'Avila-Levy *(UFRJ)*; Felipe Dias *(UFRJ)*; Rachel O. Ribeiro *(UFRJ)*; Marta H. Branquinha *(UFRJ)*; Angela H. Lopes *(UFRJ)*; Thaïs Souto-Padrón *(UFRJ)*; Rosangela M.A. Soares *(UFRJ)*; André L.S. Santos *(UFRJ)*

In this study, we report ultrastructural and growth alterations caused by cysteine peptidase inhibitors CPIs on *Phytomonas serpens*. The inhibitors antipain, leupeptin, E64 and cystatin were able to arrest cellular growth and to promote alterations on the cell morphology. E64 and cystatin did not generate detectable ultrastructural alteration. Iodoacetamide arrested the growth and induced drastic ultrastructure alterations, including disintegration of cytoplasmic organelles, swelling of nucleus and kinetoplast mitochondrion complex, which culminated with parasite death. Leupeptin and antipain induced the appearance of microvillar extensions and blebs on the cytoplasmic membrane, suggesting a shedding of the parasite surface. A 40 kDa cysteine peptidase was detected in hydrophobic and hydrophilic phases of *P. serpens* cells; this enzyme possessed similar biochemical characteristics with cruzipain, the major cysteine peptidase of *Trypanosoma cruzi*. As previously reported *P. serpens* shares common antigens with *T. cruzi*. Therefore, we have shown that anti cruzipain polyclonal antibodies recognized two polypeptides in *P. serpens*, including a 40 kDa component, partitioned on cytoplasmic and membrane-rich fractions. The presence of a cell-surface cysteine peptidase combined with morphological changes caused by leupeptin or antipain suggests the binding of the inhibitor on the cell surface cysteine enzyme and then shedding of membrane containing the inhibitor enzyme complex. This hypothesis was confirmed by ultrastructure immunocytochemical analysis, which showed the presence of the cruzipain like protein on the cell surface and on membrane fragments released by the parasites. Additionally, the involvement of these cysteine peptidases in the interaction with explanted salivary glands of the insect *Oncopeltus fasciatus* was analysed. When *P. serpens* cells were treated with CPIs and anti-cruzipain antibody, a significant reduction was observed on the interaction process. These results suggest that cysteine peptidases participate in several biological processes of *P. serpens* including cellular growth and interaction with invertebrate vector. **Financial support:** FUJB, CNPq and FAPERJ

VE11 - ANALYSIS OF UBIQUITINATED-VITELLIN IN OOCYTES OF *RHODNIUS PROLIXUS*

Mendonça, L.H. *(UFRJ)*; Salerno, A.P. *(UFRJ)*; Tomokiyo, M. *(UFRJ)*; Gomes, L.S.C. *(UFRJ)*; Cunha e Silva, N. *(UFRJ)*; de Souza, W. *(UFRJ)*; Masuda, H. *(UFRJ)*

The eggs of insects present organelles named yolk granules, which are membranous structure rich in vitellin (VT) the most abundant protein of the egg. VT is a lipoglycophosphorylated protein which provides energy and building blocks for the new embryo. Previous studies revealed the presence of different VT populations named VT1 VT2 and VT3, according to their order of elution in a DEAE column (Salerno et al, 2002). It was shown that the population named VT1 is synthesized in the follicular epithelium which surrounds the growing oocyte (Melo et al., 2000). We describe here that the VT1 population is produced by epithelial cells and are secreted to the medium already associated with ubiquitin. Using antibodies which recognize poliubiquitin and monoubiquitin it was possible to conclude that VT1 is a monoubiquitinated molecule. Immunolocalization of VT1 using an anti-ubiquitin antibody revealed the presence of VT1 at the outer layer of the oocyte. Analysis of ubiquitinated-vitellin during embryogenesis suggests that VT1 disappears during the initial phase of embryogenesis. The remaining VT seems to be re-ubiquitinated during the mid-phase of embryogenesis. The possible function of ubiquitination and re-ubiquitination of VT will be discussed. Supported by: CNPq; CAPES, FAPERJ and PRONEX.

VE12 - Soluble and membrane-bound trehalase activities in the ovary of *Rhodnius prolixus*

Santos, R. *(UFRJ)* Rosas-Oliveira, R. *(UFRJ)*; Meyer-Fernandes, J. R. *(UFRJ)*; Gondim, K. C. *(UFRJ)*

Trehalase activity has been demonstrated in different insect tissues. This enzyme catalyzes the hydrolysis of the disaccharide trehalose to two molecules of glucose. During *Rhodnius prolixus* oogenesis, glycogen reserves are produced

by the oocytes and trehalose from hemolymph could be an important substrate source for the synthesis of these carbohydrate reserves. The presence of a trehalase activity in the ovaries of *R. prolixus* was then investigated. The ovaries of *R. prolixus* showed a trehalase activity, which was maximal at seventh day after blood meal (479.9 nmoles glucose/ovary/hour). This activity slightly increased when ovaries were homogenized in the presence of the zwitterionic detergent CHAPS (3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate), indicating that trehalase is present in two forms: an overt and a latent, which can be activated *in vitro* by detergent treatment. Moreover, ovary homogenate was fractionated by differential centrifugation and soluble and membrane-bound trehalase activities were found. Both trehalase forms showed maximal activity at pH 4.5 - 6.5. For the particle trehalase, the apparent Km and Vmax were estimated to be 1.5 mM and 190 nmoles glucose/mg protein/h respectively. Isolated oocytes also showed trehalase activity, which was higher in 1.5 mm length oocytes than in 1.0 mm ones. Thus, this activity is probably involved in the uptake and hydrolysis of hemolymphatic trehalose by the follicles for glycogen synthesis during oogenesis. Supported by: CAPES, CNPq.

VE13 - Expression control of acyl-CoA-binding protein in the midgut of *Rhodnius prolixus*

Majerowicz, D. *(UFRJ)*; Grillo, L.A.M. *(UFRJ)*; Paiva-Silva, G.O. *(UFRJ)*; Gondim, K.C. *(UFRJ)*

Acyl-CoA-binding protein (ACBP) is a highly conserved 10kDa intracellular lipid-binding protein that binds straight-chain long acyl-CoA esters with very high affinity and is expressed in a wide variety of species, ranging from yeast to mammals as well as insects. Long-chain acyl-CoA esters are components in lipid uptake and transport in insects. The insect gut epithelium absorbs free fatty acids, and converts them to their CoA derivates prior to diacylglycerol formation inside cells. ACBP protects acyl-CoA esters from hydrolysis, consequently, it can function as a reserve of acyl-CoA esters by regulating their availability for various metabolic purposes. An ACBP gene has been sequenced from a midgut *Rhodnius prolixus* cDNA library. BLAST analysis showed that it has great similarity with other ACBP. Bioinformatics analysis predicts molecular weight of 10kDa, theoretical pI of 5.5, six putative phosphorylation sites, none glycosilation site and a secondary structure with four helices. ACBP expression could be detected in the midgut, fat body and ovary. Expression analysis of ACBP in the midgut by RT-PCR showed a maximum expression at second day after blood meal. We then investigated the influence of blood components in ACBP expression. Adult females were fed with tyrode buffer with or without glucose. RT-PCR results showed that there was no difference in ACBP mRNA levels in the midgut of females fed on different diets, suggesting that ACBP expression is possibly regulated by hormonal factors induced by crop extension. This study intends to investigate the role of ACBP in intracellular lipid transport in *Rhodnius prolixus* midgut. Supported by CNPq

VE14 - Proteolytic activity involved in the interaction of *Phytomonas serpens* with *Oncopeltus fasciatus* salivary glands

Alves-Silva, T.L *(IMPPG, UFRJ)*; Dias, F.A. *(IMPPG, UFRJ)*; Santos, A.L.S. *(IMPPG, UFRJ)*; Lopes, A.H.C.S. *(IMPPG, UFRJ)*

Oncopeltus fasciatus is a natural host of *Phytomonas elmasiani* and an experimental host for other trypanosomatids. *Phytomonas* species pass from the infected phytophagous insect to the plant host as the vector takes a meal. Previously we showed the ex vivo interaction between *Phytomonas serpens* and the external face of the salivary glands of the hemipteran *Oncopeltus fasciatus*, by means of scan electron microscopy. Several parasites were observed invading the basal lamina of the salivary glands, showing putative lesions in those. The involvement of proteolytic activities in the establishment of host infection by many trypanosomatids has been suggested. In this context, we decided to investigate the involvement of the proteolytic activity of *P. serpens* against salivary gland proteins of *O. fasciatus*. The extracellular and cellular proteolytic activity profiles of *P. serpens* were partially characterized and compared, by gelatin SDS PAGE, before and after the incubation of these parasites with *O. fasciatus* salivary glands. Secreted proteases presenting molecular mass of 90 kDa and optimum activity at pH 10.0 have also been observed in the culture medium of the parasites, but not in the interaction medium. A cellular cysteine protease with molecular mass ranging from 35 to 55 kDa and optimum activity at pH 5.5 in the gelatin SDS PAGE did not present this profile altered when the parasites were incubated in the presence of the insects salivary glands. However, the in vitro assay showed a high degradation of the gelatin by the protein extract of *P. serpens* at pH 5.5. This profile was confirmed by SDS PAGE analysis of the salivary gland proteins degradation after the incubation with the parasites at different pH values. These results suggest the involvement of this cysteine protease activity in the interaction of *P. serpens* with the *O. fasciatus* salivary glands. FAPERJ, CNPq and CNPq/PIBIC-UFRJ.

VE15 - CHOLESTEROL ABSORPTION, TRANSPORT AND DISTRIBUTION IN THE HEMATOPHAGOUS INSECT *RHODNIUS PROLIXUS*.

Entringer PF *(UFRJ)* Brabo LPL *(UFRJ)*; Gondim KC *(UFRJ)*

A large amount of insect lipids come from the diet. Those lipids are mainly digested and absorbed in the midgut, being exported to hemolymph associated with lipophorin, the main hemolymphatic lipoprotein. Among diet lipids, sterols are especially important to insects, since they are unable to

synthesize cholesterol by *de novo* pathway. Thus, insects depend on an exogenous source of sterol to support normal development and reproduction. In this work, neutral lipid composition of lipophorin are being characterized, as well as the distribution profile of dietary cholesterol among the tissues of the blood-feeding insect *Rhodnius prolixus* one of Chagas disease vectors. Lipophorin was purified by KBr ultracentrifugation gradient, and subjected to lipid extraction. To characterize neutral lipid composition, thin layer chromatographies were done. *R. prolixus* lipophorin is mainly composed of diacylglycerol, triacylglycerol, free fatty acid and cholesteril-ester. To characterize the cholesterol distribution pathway, *R. prolixus* females were fed with radioactive cholesterol enriched blood. Insects were dissected at different days after blood meal, lipids were extracted and radioactivity determined. There was a decrease in radioactivity present in food bolus during the days after feeding, followed by an increase of radioactivity in lipids from the midgut epithelium on third day. A significant increase of radioactive was observed in lipids from the hemolymph during the days after blood meal and the radioactivity was associated with lipophorin, as confirmed by analysis by SDS-PAGE of the fractions of the KBr gradient. These data indicated that lipophorin is the major cholesterol transporter in *R. prolixus* hemolymph. Ovaries accumulated radioactivity during the days, and radioactivity was also found in the fat body. These data suggest that *Rhodnius prolixus* also use cholesterol from the diet that is transported to different tissues by lipophorin. Other experiments are in course to better understand cholesterol metabolism in *Rhodnius prolixus*. Supported by CNPq.

VE16 - Surface metallopeptidase (Leishmanolysin-like activity) of *Herpetomonas samuelpessoai*: cleavage of different protein substrates and interaction with host

Fernanda M. Pereira *(UFRJ)*; Camila G.R. Elias *(UFRJ)*; Felipe A. Dias *(UFRJ)*; André L.S. Santos *(UFRJ)*

In a previous work, we reported that *Herpetomonas samuelpessoai* was able to release a 63 kDa metallopeptidase that had similar biochemical and immunological properties with the leishmanolysin of *Leishmania amazonensis*. In parasites belonging to the Leishmania genus, this surface peptidase is a well-known virulence factor. In this work, we aimed to identify some biochemical characteristics of the major surface metallopeptidase of *H. samuelpessoai* as well as to infer a probable function for this peptidase during the parasite-invertebrate interaction. The membrane content was obtained after parasites extraction with Triton X-114 and SDS-PAGE containing gelatin as co-polymerized substrate was used. The results showed that the peptidase had an apparent molecular mass of 63 kDa with optimal activity at acidic pH (6.0), being strongly inhibited by 10 mM 1,10-phenanthroline and hydrolyzed different proteinaceous substrates. Flow cytometric analysis provided measurements for the relative levels of *H. samuelpessoai* surface leishmanolysin-like molecules. Fluorescence microscopy corroborated with the fact that the anti-gp63 antibody recognized a similar molecule on the cell-surface of *H. samuelpessoai*. In an effort to implicate a possible role for this peptidase, we treated living parasites with different compounds before interaction with *Aedes aegypti* gut cells. When *H. samuelpessoai* cells were pre-treated with 10 mM 1,10-phenanthroline for 30 min we detected a significant decreasing in the adhesion. The pre-treatment for 60 min with two distinct anti-gp63 polyclonal antibodies (H50, H52) powerfully reduced the cellular interaction. To test the anti-gp63 specificity, an irrelevant antibody was pre-incubated with parasites, and the interaction process was very similar to that obtained with non-treated parasites. Moreover, the incubation of explanted gut for 1 h with gp63 purified protein caused a drastic inhibition in this process. Collectively, our results suggest that this metallopeptidase has an important participation on protein degradation and during the interaction with the invertebrate host.

VE17 - Activity and Localization of PATi - PAF-acetylhydrolase of *Triatoma infestans*

Assumpção, T.C.F. *(UnB)*; Motta, F.S.N. *(UnB)*; Lozzi, S.P. *(UnB)*; Teixeira, A.R.L. *(UnB)*; Santana, J.M. *(UnB)*

Most blood-sucking insects have salivary components with vasodilatory, anti-clotting, and anti-platelet aggregation activities that are capable of inhibiting hemostatic reactions of the host. Among these activities, the inhibition of platelet aggregation plays an important role during blood feeding. In accordance with this feature, we postulated that the saliva of *Triatoma infestans* would display hydrolytic activity on the platelet-activating factor (PAF). In this study we report the identification of such a PAF-acetylhydrolase (PAF-AH) activity in the saliva and salivary gland homogenates of this insect using the PAF-AH fluorogenic substrate 2-(6-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino) hexanoyl-1-hexadecanoyl-*sn*-glycero-3-phosphocoline in a Ca^{+2}-independent manner. The purification of PATi (PAF-acetylhydrolase of *Triatoma infestans*) was achieved by a two-step FPLC procedure using ion exchange and hydrophobic interaction columns. PATi was identified as a 17 kDa saliva protein on SDS-PAGE under reducing conditions. By means of peptide mass fingerprinting analysis it was possible to confirm the identity of the protein as a member of the phospholipase A2 family. This enzyme was shown to be immunogenic as it was capable to induce specific IgG antibodies in rabbit. *T. infestans* presents 3 pairs of salivary glands with different sizes and colors. They were previously described as main (D1), supplementary (D2) and accessory (D3). This protein was located in the salivary gland D2 pair by Western blot. The *T. infestans* enzyme-induced host PAF hydrolysis could be related to the inhibition of platelet aggregation and reducing the inflammation process at the site of the insect bite so as to facilitate the insect to obtain its blood meal. Supported by CNPq.

VE18 - Heme aggregation in the midgut of *Aedes aegypti* during the course of blood digestion.

Alvarenga, P.H. *(UFRJ)*; Devenport, M. *(JHU)*; Jacobs-Lorena, M. *(JHU)*; Oliveira, P.L. *(UFRJ)*

During digestion of hemoglobin blood-feeding organisms generate potentially toxic amounts of heme in their midgut. We have previously shown that most of free heme generated during blood digestion, in *Aedes aegypti*, is trapped in the peritrophic matrix (PM), however the identity of the binding molecules and the mechanisms by which this heme aggregate is formed is not known. Here we studied the heme aggregation process during the course of blood digestion by means of midgut fractionation in sucrose gradient. Twelve hours after blood meal (ABM), all the heme was found at the top of the gradient (soluble fraction). During the course of digestion, the amount of heme in the soluble fraction decreased and the heme associated to PM aggregates increased banding at higher sucrose concentrations. These results suggest that heme aggregation is a time dependent process occurring in parallel to blood digestion, probably involving PM proteins. SDS-PAGE of gradient fractions 24 hours ABM shows that some proteins, such as *Aedes aegypti* Intestinal Mucin 1, which is able to bind large amounts of heme, are exclusively localized in the heme aggregates fractions, suggesting their involvement in this process. Moreover sucrose gradient fractionation is a useful tool to isolate and study peritrophic matrix components. Supported by: HMMI, Faperj, Pronex, CAPES, PADCT and CNPq.

VE19 - Apyrase anti-haemostatic activity in the salivary gland of *Rhodnius brethesis*

Lacerda,D.P. *(UnB)*; Lozzi,S.P. *(UnB)*; Charneau,S. *(UnB)*; Teixeira, A.R.L. *(UnB)*; Santana, J.M. *(UnB)*

Silvatic *Rhodnius brethesis* is a vector of the *T. cruzi* infections in the North and Northeast regions of Brazil. The triatomine salivary gland extract was submitted to tri- and diphosphate hydrolase activity (apyrase) tests, and hydrolyses of ATP and ADP was measured by the Fisk and Subarow method. The tests were accomplished at 37 °C and pH 7.5, and the activity was strictly dependent upon Ca^{+2} ion as a cofator. The enzyme was then semi-purified in an Oligo dT Celulose® column, and the fractions that were eluted with 500 mM NaCl showed the enzyme activity. For further protein identification, salivary gland extract and eluted fractions under non-reducing conditions (no boiling and no DTT) were separated by SDS-PAGE. The 65 kDa protein band displaying apyrase activity was then identified by calcium phosphate precipitation method, after gel incubation with either ADP or ATP. The protein was transferred to a nitrocellulose membrane in a semi-dry system and incubated with anti-apyrase serum raised in rabbit immunized with the *Triatoma infestans* apyrase. Although the anti-apyrase antibody showed antigen specificity it did not recognize the *R. brethesis* apyrase-counterpart. Nevertheless, the platelet aggregation assay *in vitro* showed that 8 μg of salivary extract of *R. brethesis* completely abolished aggregation of platelets equivalent to 1 ml of human blood. This functional assay confirms the anti-haemostatic activity, and this work appears to be the first description of an apyrase in the salivary gland *R. brethesis*. Supported by CAPES and CNPq.

VE20 - Lysophosphatidylcholine depletion induces the down-regulation of nitric oxide synthase during Trypanosoma rangeli infection in Rhodnius prolixus salivary glands.

Lopes, F.G. *(ICB-CCS-UFRJ)*; Mesquita,R.D. *(ICB-CCS-UFRJ)*; Ximenes, A. *(ICB-CCS-UFRJ)*; Machado, E. A. *(IBFCCF-CCS-UFRJ)*; Atella, G.C. *(ICB-CCS-UFRJ)*; Silva-Neto,M.A.C. *(ICB-CCS-UFRJ)*

Trypanosoma rangeli is the sole protozoan parasite that reaches and penetrates *Rhodnius prolixus* salivary glands. *Rhodnius* saliva contains several molecules including nitric oxide (NO) bound to a set of proteins named nitrophorins.Recently, our group has demonstrated the presence of a novel antihemostatic compound in *Rhodnius* salivary glands the bioactive phospholipid, lysophosphatidylcholine (LPC) (Golodne et al, 2003). Furthermore, we have also shown that *T.rangeli* actively removes phospholipids from culture media (Folly et al 2003). In fact, invaded salivary glands exhibit a low content of LPC when compared to controls. This effect parallels the observations by Garcia et al. (1994) who demonstrated that *T.rangeli* infection causes a decrease in the levels of a NO derivative, nitrite. We then investigated if LPC levels could modulate NO synthesis and storage. *Rhodnius* nitric oxide synthase (RpNOs) activity is kept at a basal level after blood feeding and NO synthesis is two fold increased after imaginal moulting. Insects were infected with *T.rangeli* and salivary glands were dissected 1, 7 and 21 days later. RpNOs activity and levels were then assayed in infected salivary glands. A 50% decrease in RpNOs activity was observed in infected animals when compared to uninfected controls. Imunohistochemistry was performed in infected salivary glands using anti-NOS antibodies. RpNOs was only immunolocalized in control glands. Therefore, parasite invasion decreases NO production through a pathway which down regulates RpNOs expression. To check for the role of LPC on RpNOS expression we have injected LPC in fourth instar nymphs seven days after feeding. RpNOs activity in salivary glands was three fold increased when compared to controls. In future experiments our group will supplement infected animals with LPC in order to determine if this phospholipid may recover RpNOs activity and decrease parasitemia in infected glands. Supported by CNPq (PIBIC e PGI) , IFS, FAPERJ,PADCT.

VE21 - Dengue virus infection and tyrosine phosphatases in mosquito cells.

Bretas, J. *(UFRJ)*; Senna, R. *(UFRJ)*; Conceição, T.M *(UFRJ)*; Da poian A.T *(UFRJ)*; Silva-Neto, M.A.C. *(UFRJ)*

Dengue is transmitted by *Aedes aegypti* and is the arthropod-borne disease more prevalent in human population, with about 50 million new cases per year. Despite this huge impact the mechanisms of transmission of such disease by mosquitoes are still unknown. One of the most important mechanisms of cellular signaling in eukaryotes is the reversible phosphorylation of proteins. Such mechanism has an important role in the regulation of cellular proliferation and differentiation, gene expression among others. The objective of this study is to identify the mechanisms involved on cell signaling in the dengue infection in the insect vector. So far we have used cultured cells of *Aedes albopictus* (C6/36). The present work evaluates the effect of the infection on the tyrosine phosphatase activity. Infected cell cultures with dengue virus type II, we observed that infected cells presented a lower phosphatase activity (60 %) than control cells. Such effect was evaluated in intervals ranging from 10 minutes to 12 hours. To determine the involvement of such enzyme in a possible cascade of signaling events in mosquito immune system the following experiment was carried out. Cells were incubated in the presence of LPS, a polysaccharide from the membrane of gram-negative bacteria, and an agonist of Toll pathway. Curiously, in such conditions we observed an increase of the phosphatase activity. Such results suggested for the first time the involvement of a tyrosine phosphatase in mosquito immune response. They also suggest that the mechanisms of viral infection might manipulate intracellular signaling cascades of the vector in a different fashion of that described in studies with classic agonists of the immune system such as LPS. Supported by for CNPq, FAPERJ, PADCT.

VE22 - Further investigation on the receptor-ligand involved in the interaction of *Phytomonas serpens* with salivary glands of *Oncopeltus fasciatus*

Dias, F.A. *(IMPPG, UFRJ)*; Santos, A.L.S. *(IMPPG, UFRJ)*; Lery, L.M.S. *(IBCCF, UFRJ)*; Bisch, P. *(IBCCF, UFRJ)*; Saraiva, E.M. *(IMPPG, UFRJ)*; Souto-Padrón, T. *(IMPPG, UFRJ)*; Lopes, A.H.C.S. *(IMPPG, UFRJ)*

The hemipteran phytophagous *Oncopeltus fasciatus* is a natural host of *Phytomonas elmasiani* and an experimental host for other species of trypanosomatids. The plant-sucking insects act as intermediate hosts, being the plants the main hosts in the biological cycle of *Phytomonas* species. These parasites pass from the infected phytophagous insect to the plant host as the vector takes a meal. In this context, the invasion of the salivary glands of these vectors is one of the most important events for the life cycle of *Phytomonas* species. In the present study we observed, by means of scanning electron microscopy, the ex vivo interaction between *P. serpens* and the external face of salivary glands of *O. fasciatus*. This binding seems to occur through a 130 kDa protein located at the salivary gland basal lamina. The mass spectrometry of the trypsin-digest of this protein matched 23% of human laminin beta 3 chain precursor sequence by the digested peptides. In addition, polyclonal antibodies raised against human laminin were able to recognize this protein, through immunoblotting. To investigate the parasite receptor for the 130 kDa protein, we incubated blots of parasite proteins with total proteins of salivary glands pretreated with NHS Lc biotin, the purified biotinylated 130 kDa protein or biotinylated human laminin. The profile of reactive proteins from the parasite was very similar in the different systems. Supported by: CNPq, FAPERJ and CNPq/PIBIC-UFRJ.

VE23 - Effects of malaria infection on vitellogenesis in Aedes aegypti

Feitosa, FM *(USP)*; Marinotti, O *(UCI)*; de Bianchi, AG *(USP)*; de Lara Capurro, M *(USP)*

Plasmodium infected mosquitoes are important as a model for the study of pathogen- insect interaction. We present a preliminary transcriptome and a cDNA macroarray analysis of Ae. aegypti vitellogenic fat bodies. The trancriptome of the mosquito under various physiological stages as well as the cDNA amounts of isolated tissues are important additions to the complete genome description. We focus here on the transcriptome of Ae. aegypti vitellogenic fat body. This organ participates in multiple biochemical functions including protein, amino acid, lipid and carbohydrate synthesis and storage, xenobiotic detoxification, and immune response. A total of 590 randomly selected cDNAs were sequenced and assembled into 262 clusters, based on their similarities. The putative translated proteins were classified into three categories: oogenesis-related (15%), housekeeping products (65%) and products with unknown cell localization and function (20%). A partial macroarray analysis was done using 57 of those transcripts. The membranes were hybridized with cDNAs made from total RNA samples obtainde from (1) males, (2) sucrose fed females, (3) females 24 hours after blood feeding and (4) females 24 hours after blood feeding, infected with Plasmodium gallinaceum. The results show that 25 transcripts increase in amount after blood feeding when compared to sucrose fed females or males. Supported by FAPESP

VE24 - Dipeptidyl peptidase of *Trypanosoma brucei* and *Trypanosoma cruzi*

Bastos, I. M. D. *(LMPDC)*; Motta, F. S. N. *(LMPDC)*; Girard, D. *(MNHM)*; Charneau, S. *(UnB)*; Santana, J. M *(LMPDC)*; Teixeira, A. R. L. *(LMPDC)*; Grellier, P. *(MNHN)*

Dipeptidyl peptidases (DPP) are ubiquitous enzymes. Many DPPs have been described and the most known among them is the dipeptidyl peptidase IV, a type II multifunctional cell surface protein that belongs to the prolyl oligopeptidase family of serine proteases. DPP IV specifically removes dipeptides from the N-terminus of peptides with a Pro, Hyp or Ala at the penultimate position. DPP IV substrates include certain chemokines, growth factors (e.g. growth hormone, growth hormone-releasing factor, glucagon-like peptides 1 and 2) and neuronal and vasoactive peptides including neuropeptide Y, peptide YY and substance P. In this work, we present the identification of a dipeptidyl peptidase of *Trypanosoma brucei* (DPPTb) and *Trypanosoma cruzi* (DPPTc). Their genes were isolated by PCR using primers designed from *T. brucei* ESTs sequences, which match the human DPP IV. However, the sequences of DPPTb and DPPTc display similarity mainly with human DPP8 and DPP9. DPPTc shares 55% identity with its *T. brucei* counterpart, DPPTb. Active recombinant DPPTb was expressed in insect cells using the baculovirus system. Immunofluorescence experiment, using anti-DPPTb antibodies, revealed that native DPPTb is located within vesicles in the cytoplasm of procyclic forms of. *T. brucei*. Supported by CAPES.

VE25 - Prolyl oligopeptidase of *Trypanosoma brucei* (POPTb) hydrolyzes hormone peptides and is involved in parasitic growth

Bastos, I. M. D. *(LMPDC)*; Santana, J. M. *(LMPDC)*; Teixeira, A. R. L. *(LMPDC)*; Grellier, P. *(MNHN)*

Proteases play important roles in many processes of the biology of parasites including interactions with their hosts. We have reported that prolyl oligopeptidase of *Trypanosoma cruzi* (POPTc80) is associated with the entry of trypomastigotes into mammalian host cells. In this study, the gene coding for prolyl oligopeptidase of *Trypanosoma brucei* (POPTb) was identified and characterized. It is represented by a single copy per haploid genome of the parasite and its deduced amino acid sequence shares 77% identity with POPTc80. Secondary structure predictions demonstrated that POPTb shows the highly preserved secondary structure composition and arrangement of prolyl oligopeptidases. Active recombinant POPTb, produced in *E. coli*, displayed enzymatic activity on peptides containing Pro at P1 position and collagen at a slightly alkaline pH. Its enzymatic activity was highly sensitive to POPTc80 inhibitors. Furthermore, these inhibitors arrested growth of procyclic and bloodstream forms of *T. brucei* in a dose-dependent manner. The specific hydrolysis of several hormone peptides and neuropeptides is the most important feature of prolyl oligopeptidases. As hormone alterations are closed associated to symptoms observed in African trypanosomiasis, we investigated the ability of POPTb to cleave peptides such as bradykinin, neurotensin, LHRH, β-endorphin and TSH. POPTb readly hydrolyzed all of these peptides, always at the C-terminal of proline. These results might indicate that POPTb, released in the blood by parasite lysis, could be associated to patogenesis of African trypanosomiasis. Supported by CAPES.

VE26 - *Aedes aegypti* metabolizes heme into a soluble and antioxidant biliverdin

Pereira, L.O.R. *(UFRJ)*; Pires, R.S. *(UFRJ)*; Almeida, I.C. *(USP)*; Oliveira, P.L. *(UFRJ)*; Paiva-Silva, G.O. *(UFRJ)*

In the midgut of hematophagous animals, blood digestion results in the release of the hemoglobin prosthetic group (heme), a molecule with a high oxidative potential. Nevertheless, these organisms have shown a wide world distribution. It requests several defense mechanisms against this oxidative challenge. One of these strategies is the enzymatic degradation of heme that has already described in several organisms, including plants, bacterias and mammals. This reaction is catalyzed by heme oxygenase and results in CO, ferrous ion and biliverdin IX alpha. According to the enormous epidemiologic relevance of the mosquito *Aedes aegypti*, we intended to identify and characterize heme degradation pathway in this insect. During blood digestion, huge amounts of heme are released, resulting in an intense production of a green pigment, by midgut epithelium cells. This pigment is secreted to the intestinal lumen, reaching maximum values 24h after blood meal, matching the critical phase of digestion. At the end of this process, this pigment is completely excreted by the mosquito. By HPLC in a reverse phase C18 column, with an acetonitrile gradient, this pigment was purified. Structural characterization by ESI-MS revealed that mosquito pigment is an alpha isomer of biliverdin modified by a peptide-like binding of two glutamine residues. The biglutaminated biliverdin production pathway was also identified. It is composed by the heme degradation step, catalyzed by heme oxygenase expressed in the midgut epithelium, followed by two subsequent additions of a glutamine residue. We also investigated the antioxidant capacity of *A. aegypti* biliverdin. It was capable to prevent B-phycoerythrin oxidation by free radicals. This protection occurs in a dose dependent manner, as observed for biliverdin IX alpha and trolox, a well-known hydrosoluble antioxidant molecule. This finding revealed an important physiological strategy of heme metabolism in this insect. Supported by CNPq, FAPERJ, PRONEX, PADCT and HHMI

VE27 - Flexibility between α/β-hydrolase and β-propeller domains is required for access of substrates to the catalytic site of the *Trypanosoma cruzi* POP Tc80.

Bastos, I. M. D. *(LMPDC)* Motta, F. S. N. *(LMPDC)*; Lima, M. M. *(LMPDC)*; Maigret, B. *(LCT)*; Grellier, P. *(MNHN)*; Teixeira, A. R. L. *(LMPDC)*; Schrével, J. *(MNHN)*; Santana, J. M. *(LMPDC)*

Prolyl Oligopeptidase is a family of enzymes with specificity for peptide bonds at the carboxyl side of proline residues. We have characterized an 80 kDa prolyl oligopeptidase of *Trypanosoma cruzi* (POP Tc80), and demonstrated that it is involved in the process of parasite entry into mammalian cells. In contrast with other POPs, POP Tc80 is capable of hydrolysing large substrates, such as fibronectin and native collagen. It seems that the general structure of this enzyme, based on a crystallized porcine POP, presents a catalytic α/β-hydrolase domain and a non-catalytic β-propeller domain. On the basis of our docking analysis, we suggest that access of the triple-helical collagen to the catalytic pocket of POP Tc80 takes place in the vicinity of the α/β-hydrolase and β-propeller. Then, the substrate entrance to the active site of the enzyme is most likely through a side opening situated between both domains. To test this hypothesis, three site-specific mutagenesis independent experiments were performed to introduce cysteine residues into the α/β-hydrolase domain at favorable positions (Ser591-Cys591; Asp471-Cys471; Ala588-Cys588). This would allow the formation of disulfide bonds with Cys255 of propeller domain, thus blocking the access of substrate to the enzyme catalytic site. Experimentally, oxidation of mutant POP Tc80 caused a decrease in its enzymatic activity. Reduction of these mutants restored their activity to a level, which is comparable to that of the control. These experiments demonstrated that flexibility between the two domains of POP Tc80 is required for the entrance and posterior cleavage of the substrates. Supported by CNPq

VE28 - Effects on cathepsin B and cathepsin D activities in midgut of *Rhodnius prolixus* by *Trypanosoma cruzi* infection

Borges EC *(Fiocruz)*; Machado EMM *(Fiocruz)*; Garcia ES *(Fiocruz)*; Azambuja P *(Fiocruz)*

Hematophagous hemipterans produce lysosomal acidic proteases to digest their diet, being the cathepsins B and D the major ones in *Rhodnius prolixus* midgut (Houseman & Downe 1982). Garcia et al. (1995) suggested that *Trypanosoma cruzi* could interact with the insect midgut digestive enzymes and digestion products, possibly modulating the parasite metabolism and its infectivity. The aim of the present work was to investigate if cathepsins B and D dynamic activities were affected by the presence of the parasite in the midgut of *R. prolixus*. Cathepsin B and D specific activities were estimated in midgut homogenates from *R. prolixus*, uninfected and experimentally infected with *T. cruzi*, at different days after blood ingestion. No enzyme activity was found in the anterior midgut (stomach) and rectum. In the posterior midgut (intestine), enzyme activities were found both in lumen and wall. In starved uninfected insects cathepsin D activity was high, decreasing to a constant rate at 1 to 15 days after feeding. Cathepsin B activity was also high in starved insects, especially in the lumen. In the wall, cathepsin B activity was high at the 1st, 3rd and 9th day post-feeding. Insects infected with *T. cruzi* presented alterations in both enzymes dynamic activities: cathepsin D increased at the 1st and 3rd days after blood meal, while cathepsin B decreased in all evaluated days. We suggest that *T. cruzi* modulates the activity levels of these proteinases in the intestine of *R. prolixus*, increasing the cathepsin D activities and decreasing cathepsin B. The role of these enzymes in the parasite development and metacyclogenesis remains unknown and is under investigation. Supported by Fiocruz, FAPERJ, CNPq, Volkswagen Foundation.

VE29 - CHARACTERIZATION OF TRYPSINS IN *LUTZOMYIA LONGIPALPIS*

Telleria E L *(FIOCRUZ)*; Batista L M *(FIOCRUZ)*; Ortigão-Farias J R *(FIOCRUZ)*; Barroso R M *(FIOCRUZ)*; Traub-Cseko Y M *(FIOCRUZ)*

Leishmaniasis is widely distributed in the Americas, and the number of cases has increased over the last 20 years. The parasite is transmitted to the mammal hosts by Phlebotomine sand flies bites. *Lutzomyia longipalpis* is the main vector of *Leishmania chagasi*, which causes visceral disease in Brazil. We are studying molecular aspects of blood feeding and interaction between *Leishmania* parasites and their insect vector host, intending to characterize specific molecules that might have a significant role in these processes. These molecules may be used as potential targets for the development of new strategies in the fight against the spread of leishmaniasis. Midgut-specific proteins like trypsin, chymotrypsin and chitinase are potential targets for the development of transgenic insects unable to transmit diseases, and have been used for this purpose in mosquitoes. We have isolated two trypsin cDNAs (Trip-1 and Trip-2) from an expression library of blood-fed *L. longipalpis* midgut, similar to other insect genes. Trip-2 cDNA is completely sequenced. RT-PCR shows that Trip 2 is expressed 6 hours after blood ingestion and is probably involved in blood digestion. Trip-1 is almost completely sequenced. When both sequences are available specific primers will be designed to evaluate the expression of each gene by RT-PCR at different times after a bloodmeal. It is known that mosquitoes have early and late trypsins, with different timing of expression. We have cloned a fragment of Trip-2 in the expression vector pET 28a and a recombinant protein was produced. A polyclonal serum was obtained, that is being used in functional studies. These antibodies are being used in imunolocalization studies using dissected guts or cut whole insects, and Western blots to investigate the timing of protein expression.

VE30 - Effects of physalin B on the immune reactions of *Rhodnius prolixus* larvae infected with *Trypanosoma rangeli.*

Garcia, ES *(IOC)* Castro, DP *(IOC)*; Ribeiro, IM *(FAR)*; Tomassini, TCB *(FAR)*; Azambuja, P *(IOC)*

The biological life cycle of *Trypanosoma rangeli* in the invertebrate host, *Rhodnius prolixus*, depends on the invasion of the hemocoel and completes its development in the salivary glands, resulting in an inoculation transmission. Recently, physalins, seco-steroids obtained from *Physalis angulata* L., inhibited macrophage activation, nitric oxide production and caused lipopolysaccaride-induced death in a murine model of endotoxic shock. In the present paper, we tested physalin B as immunomodulatory compound in 5th-instar larvae of *R. prolixus* that were systemically infected with the protozoa *T. rangeli* strain H14 and Choachi. The effective dose of physalin B, which inhibited 50% of the volume of the blood ingested (ED50), was of 15.2 mg/ml of meal. Ecdysis processes and mortality in larvae treated orally with physalin B at doses ranging from 1 to 10 mg/ml were similar to those observed in untreated insects. However, *R. prolixus* larvae previously fed on blood containing 1.0, 0.1 and 0.01 mg of physalin B/ml, showed mortality rates of 78.1, 54.3 and 12.7% respectively 6 days after inoculation of *T. rangeli* (1 x 103 parasites), whereas only 7.2% of mortality was observed in the control group injected with sterile culture medium. Insects fed with blood treated with physalin B (0.1 mg/ml) and 1 x 106 parasites/ml strain Choachi had high level of parasitemia on posterior medium crop after 17 days of infection compared with control, treated only with parasites. The insects treated with physalin B (0.1 mg/ml) and inoculated with protozoa did not modify the phenoloxidase (PO) activity and hemocyte counting in the hemolymph. However, the physalin B treatment caused a significant inhibition of hemocyte microaggregation and nitric oxide production and enhanced the parasitemia in the hemolymph. These results demonstrate that physalin B from *P. angulata* is a potent immunomodulatory substance for the bloodsucking insect, *R. prolixus*.

VE31 - EMBRYONIC CELLS OF TICK *BOOPHILUS MICROPLUS* AS MODEL FOR STUDIES ON THE IMMUNE RESPONSE

Eliane Esteves *(USP)*; Aline Harumi Fukuzawa *(USP)*; Daniel Macedo Lorenzini *(USP)*; Bruno C Vellutini *(USP)*; Leandro N Pressinotti *(USP)*; Timothy J Kurtti *(USA)*; Jesus A Ferro *(UNESP)*; José R M C Silva *(USP)*; Sirlei Daffre *(USP)*

Ticks are obligatory blood-sucking arthropods, being one of the most important vectors of both human and animal diseases. We have previously characterized some cellular and humoral immune reactions in the cattle tick *Boophilus microplus*: *i.* phagocytic activity and production of reactive oxygen species (ROS) by hemocytes upon an *in vitro* microbial challenge *Exp Parasitol*, 2001, 99: 66); and *ii.* four antimicrobial peptides (AMP) from the midgut contents *J Biol Chem*, 1999, 274: 25330) and hemolymph *Devel Comp Immunol*, 2004, 28: 191). We are currently interested in studying the *B. microplus*-pathogens relationship using an immune approach. Due to the feeding specificity of *B. microplus* for bovine host blood, it is difficult to offer an artificial meal to these animals. Therefore, we have started working on the immune response using a culture of embryonic cells (BME 26) *Exp. Appl. Acarol.*, 1989, 7 :219) as model. Firstly the ultrastructure of these cells has been investigated by means of transmission electronic microscopy. We observed a large number of cells containing granules. Interestingly these cells play some immune functions, such as phagocytic activity and expression of two AMP (microplusin and defensin) coding genes. A cDNA library was constructed and the sequencing of about 800 clones reveal several abundant transcripts related to different functional classes. Among them, the most abundant is ferritinn, which is well-known involved with both iron transportation and defense functions. With the aim of characterizing the relevance of some transcripts related to the tick immune system, we intend to silence their coding genes by RNAi and evaluate the survival of the BME26 cells upon challenge with different pathogens.

VE32 - The ultrastructure of the fat body of the mosquito AEDES AEGYPTI

Martins, G.F. *(CPqRR-Fiorcruz-MG)*; Serrão, J.E. *(DBG-UFV)*; Pimenta, P.F.P. *(CPqRR, Fiocruz-MG)*

Fat body of insects is involved in the intermediary metabolism and in the regulation of storage and transport of many precursors to be released to the hemolymph. In this work, we investigate the ultrastructure of the *A. aegypti* fat body. We used newly-emerged females, 18 days old sugar-fed females and 18 d old blood-fed females (blood fed in the 14th day and dissected four days after blood meal). Abdominal fat body was fixed in 4% glutaraldehyde in 0.1M caccodylate buffer with 7% sucrose, post-fixed in 1% osmium tetroxide in 0.8% potassium ferrycianide and 0.1M caccodylate buffer, pH 7.2. The samples were dehydrated in acetone series and embedded in Epon. Ultrathin sections were contrasted with uranyl acetate and lead citrate to be analyzed in a Zeiss EM 109 transmission electron microscope. Fat body trophocytes of *A. aegypti* present a globular shape and a nucleus squeezed between numerous lipid inclusions and proteins granules that disappear during aging and after blood meal. Trophocyte cytoplasm is plentiful of glycogen and ribossomes. Mitochondria and rough endoplasmic reticulum (RER) are mainly located around the nucleus and along cell membrane. After blood meal, RER vesicles enlarge and the number of mitochondria increases, suggesting an increase of cell activity, as well as, some organelle autolysis. Cytoplasm of sugar-fed female trophocytes becomes disorganized, full of lipid droplets, with lesser amount of ribossomes and glycogen, and RER and mitochondria almost disappear. Oenocytes present in-

vaginations that are more numerous facing the hemolymph. Their cytoplasms are dense in comparison to trophocytes, rich in mitochondria and lysosome-like structures. In both cell types, developed nucleolus can be seen. Our study indicates that cell remodeling is involved in functional changes of fat body during aging and after blood meal.Supported by: FAPEMIG

VE33 - Fat body morphology of five mosquito species

Martins, G.F. *(CPqRR-Fiorcruz-MG)*; Pimenta, P.F.P. *(CPqRR-Fiorcruz-MG)*

Fat body of insects is a multifunctional organ for storage and release of the hemolymph components. In this work we study the fat body morphology of *Aedes aegypti*, *Ae.albopictus*, *Ae.fluviatilis*, *Culex quinquefasciatus* and *Anopheles aquasalis*. Fat bodies of new-emerged males and females were fixed in 4% glutaraldehyde in 0.1 M caccodylate buffer and post-fixed in 1% osmium tetroxide. Samples were dehydrated in acetone, dried by critical point device and gold coated for observation under the scanning electron microscope. Other samples were embedded in historesin. Two micrometer sections were stained with toluidine blue. For protein and uric acid detection, sections were stained respectively, with trichrome of Gomori and silver-hexamine solutions. Fat bodies of mosquitoes form a great cell mass underneath integument along body cavity. They are formed by two cell types: the trophocytes which have a globular shape, a pleomorphic nucleus and a cytoplasm rich of lipid inclusions and protein granules; and the oenocytes with a cytoplasm homogenously stained for proteins. This observation suggests an intense protein synthesis and storage in both cells. Under SEM, the fat body is seen as a continuous layer scattered beneath integument with lobes projecting into abdominal cavity. The fat body is externally covered by a basal lamina that forms invaginations between the cells. Oenocytes have folds on cell surface that are not seen in trophocytes. As revealed by silver-hexamine method, *Anopheles* trophocytes present cytoplasmic black granules that probably correspond to uric acid. Urocytes or urate cells have not yet been reported in mosquito fat body. However uric acid accumulation in trophocytes may compensate this lacking. Uric acid storage could be an adaptation for larval saline environment, since uric acid and salts are stored as urates. In addition, *Anopheles* trophocytes present spherical cell projections that may correspond to protein or lipid storage in their inner part.

VE34 - THE INVASIVE CAPACITY OF A NEW RHABDITIDA (NEMATODA) INFECTING *Lutzomyia longipalpis*

Macedo, C.G. *(CPqRR-Fiocruz-MG)*; Barreto, D.Z. *(CPqRR-Fiocruz-MG)*; Pimenta, P.F.P. *(CPqRR-Fiocruz-MG)*; Secundino, N.F.C *(CPqRR-Fiocruz-MG)*

Lutzomyia longipalpis is a sandfly vector of important pathogens worldwide and are the only proven vector of *Le. chagasi* transmitting human visceral leishmaniasis. Several entomoparasites have been described in phlebotomines, including virus, bacteria, protozoa, fungi, nematodes and mites, some of which are capable of killing the insect host. The objective of this study was to recognize the morphology and the invasive capacity of an entomoparasite nematode infecting *Lu. longipalpis*. Entire sandflies were dissected between strikes of the last abdominal segment and revealed the presence of nematodes. These worms were encountered in the abdominal cavity of both sexes. The Scanning Electron Microscope showed several eggs in different embryonic stages. The nematode eggs were deposited in the abdominal cavity and in the region located between the exoskeleton and fat body cells. Distinct forms of nematodes were observed, such as, eggs, juvenile larvae and adults. The juvenile forms were filiform. In the eggs, it was possible to distinguish details of the thick chorion including a rough membrane around the body of the nematode. Large nematodes (sexual or adult forms) found inside cyst-like structures or free among the larvae in the haemocoel. In heavy infections, the histology showed nematode larvae inside the whole abdomen, or other regions of the sandfly, such as, leg and thoracic muscles. These observations demonstrate the invasive capacity of this nematode. The infection with this nematode may difficult the sandfly life cycle reducing the number eggs or the ability of flying. Studies are in progress to formally identify and describe this new Rhabditida (Nematoda). Its ability to infect and kill phlebotomines makes it a potential candidate for biological control of species, which transmit human pathogens like *Leishmania* parasites.

VE35 - EGG BUSTERS OF MOSQUITO VECTORS: A SCANNING ELECTRON MICROSCOPIC STUDY

Silva, T. A. *(CPqRR/Fiocruz)*; Pimenta, P.F.P. *(CPqRR/Fiocruz)*; Secundino, N.F.C. *(CPqRR/Fiocruz)*

Mosquitoes are vectors of several human diseases, such as, malaria, dengue and filariasis. During mosquito egg hatching, the chorion rupture is made by a specialized structure on the larvae called egg buster. In mosquitoes, the egg buster is present only at the first larval stage being observed as a small and median cuticle projection, placed on the dorsal-central region of the head as observed by optical microscopy. The larval movement provokes a crack in the eggshell with the egg buster located in the dorsal head. In this study,

we used scanning electron microscopy (SEM) to describe details of the egg busters of four mosquito species that are vectors of pathogens. First larvae of *Aedes albopictus*, *Aedes fluviatilis*, *Anopheles aquasalis* and *Culex quinquefasciatus* were collected from laboratory colonies and fixed in 4% glutaraldehyde in 0.1 M caccodylate buffer and post-fixed in 1% osmium tetroxide. The fixed samples were dehydrated in a series of acetone, dried in a critical point device and coated with gold particles in order to be observed on the SEM. All mosquitoes present the egg buster located inside a dorsal head depression that differs morphologically according to the species. In *Ae. albopictus*, the egg buster is a spine-like structure inside a head depression that resembles a droplet-like structure. In *Ae. fluviatilis*, the egg buster has an irregular surface with many protuberances. In *An. aquasalis*, the egg buster presents a triangle-like structure with a tip on the top. Finally, the egg buster of *C. quinquefasciatus* has a mid-moon shape. In conclusion, this study describes and differentiates the anatomy of the egg busters in different mosquito species, which represent three distinct genera. Supported by: FAPEMIG

VE36 - MORPHOLOGICAL CHARACTERISTIC AND SENSORIAL ORGANS OF THE ANTENNA OF FEMALES OF Phlebotomus papatasi (DIPTERA: PSYCHODIDAE) BY SCANNING ELECTRON MICROSCOPY

Bahia-Nascimento, AC *(CPqRR-FIOCRUZ-MG)*; secundino, NFC *(CPqRR-FIOCRUZ-MG)*; Fernandes, FF *(UFG)*; Pimenta, PFP *(CPqRR-FIOCRUZ-MG)*

Phlebotomus papatasi is a Phlebotomine sand fly involved in the transmission of cutaneous leishmaniasis in the Old World. We analyzed the ultra structural aspects of the antennae focusing in morphology and sensorial organs of female sandfly using scanning electron microscopy. Our analyses showed the presence of a pair of filliform antennae that are formed by sixteen segments: the scape (first or basal segment) is triangular in shape; the pedicel (second segment) has a globular form, and after that, there are fourteen filliform flagellomeres. The first flagellomere is longer than the others. The second to the eleventh flagellomeres are very similar. The twelfth, thirteenth and the last one are less globular and rugous. Smaller non-innervated spinules, the *microtrichia*, were observed in all extension of the antennal segments, but the quantity diminishes from the twelfth to the fourteenth flagellomeres. Our results also showed the presence of seven different sub-types of sensilla: *campaniforme*, *chaetic*, *coeloconic*, *smaller trichoid*, *pointed tip trichoid*, *blunt tip trichoid* and *squame*. The *campaniforme sensilla* were observed on the pedicel. The *squame* were present on the pedicel and the first flagellomere. The *smaller trichoid sensilla* were found on the scape, pedicel and on the apex of the nineteenth and the thirteenth flagellomeres. The *trichoid blunt sensilla* were observed on all the segments, although, on the last one, they were only present on the base of the segment. The *chaetic sensilla* were found on the lateral of each of the first thirteenth segments. The *coeloconic sensilla* appeared from the twelfth to the fourteenth flagellomere. The *pointed tip trichoid sensilla* appeared on these same segments and on the eleventh flagellomere. These observations represent a study that should be used for the fundamental base to posterior taxonomic consideration and to knowledge about the physiology and behavior of this species in a natural environment.

VE37 - STUDY OF SENSORIAL ORGANS OF THE LARVAE AND ANTENNA OF FEMALES OF Lutzomyia intermedia (DIPTERA: PSYCHODIDAE) BY SCANNING ELECTRON MICROSCOPY

Bahia-Nascimento, AC *(CPqRR-FIOCRUZ-MG)*; Secundino, NFC *(CPqRR-FIOCRUZ-MG)*; Fernandes, FF *(UFG)*; Pimenta, PFP *(CPqRR-FIOCRUZ-MG)*

Lutzomyia intermedia is a New World phlebotomine sand fly involved in a transmission of American Cutaneous Leishmaniasis in South American countries including in Brazil. Details of the insect sensorial organs, sensilla, have been used for taxonomic studies in larva and adult. In this work, we analyzed the ultra structural aspect of the sensilla in the *L. intermedia* using the scanning electron microscopy. On the larvae, we found different subtypes of *trichoid sensilla*. The *brush-like trichoid sensilla* localized along dorsal and lateral parts of the larvae and behind the antennae on the top of the head. The *weakly brush-like trichoid sensilla* are found on the middle of the head. The *straight trichoid sensilla* are observed on the buccal apparatus. They are disposed in a line in front of the *weakly brush-like trichoid sensilla*. The *curved long trichoid sensilla* are found in each extremity of pseudolegs. We also found five sensilla inserted in the antenna. One of these sensilla presented porous similar to the one in the caudal filaments. On the antennae of adult female, we found *microtriquias* and seven sub-types of sensilla. The *coeloconic* and the *pointed tip trichoid sensilla* appeared from the twelfth to the fourteenth flagellomeres. The *chaetic sensilla* were found from the first until the thirteenth flagellomeres. The *smaller trichoid sensilla* were observed on the scape, pedicel and on the apex of the nineth, eleventh and thirteenth flagelomeres. The *trichoid blunt sensilla* were observed in all segments. The *squame sensilla* were found on the pedicel and the first flagellomere. Finally, *campaniforme sensilla* were found only on the pedicel. This study should be used to understand the biology and physiology of the different stages of this insect; and this information should assist us in the development of control strategies for this important vector.

VE38 - RESEARCH ON THE INCIDENCE OF PHYTOPHAGUS HEMIPTERA INFECTED WITH LOWER TRYPANOSOMATIDS IN THE WEST REGION OF SÃO PAULO STATE, BRAZIL.

Neris, M. D *(FAI)*; Guimarães, R. S. O *(FAI)*; Azolini, M. A *(FAI)*; Bucke, M. H. S *(FAI)*; Coverloni, A *(FAI)*; Bovi, E. C. V *(FAI)*; Fernandes, S. L. O *(FAI)*; Lott, A. R *(FAI)*; Erler, R. R. *(FAI)*; Oliveira D *(FAI)*

The Trypanosomatidae family ranges from flagellated, mono or heteroxenic protozoa, parasites of plants to invertebrate and vertebrate animals. Among the invertebrate animals, the phytophagous hemiptera are hosts of many species of lower trypanosomatids. During the first century of studies on trypanosomatids only 350 species of insects were identified as hosts of trypanosomatids. It can be estimated that from 1 million species of insects known no more than 2500 have been studied by parasitologists. Thus, only a minority of insects collected from a limited number of locations has been examined in search for trypanosomatids. Recent studies also describe about 100 species of flagellates found in different species of plants from which feed the hemipteras. Trypanosomatids found in coconut tree, palms of olive oil, coffee plant, cassava and Alpinia purpurata are responsible for lethal phytopathologies. Adults and nymphs of phytophagous hemiptera infected with trypanosomatids feed on developing seeds and fruits, causing colour loss, rottenness and falls of the fruits and it generates economical damages because of a decrease in the agricultural production. Recent work proposes the use of the own trypanosomatids in the thumbtacks biocontrol, once commensal intraespecific trypanosomatids can be pathogenic on interespecific populations of insects. The present research has as objectives: a) the study of incidence of the infections with lower trypanosomatids on phytophagous hemiptera in the west region of São Paulo State Brazil; b) the isolation of the tripanosomatids starting from the dissection of the insects; c) the execution of tests of susceptibilities from different phytophagous hemiptera to different isolated trypanosomatids and also to the isolated 563DT (Leptomonas/UEL) isolated from Euchistus herus and pathogenic for Leptoglossus zonatus. Keywords: Trypanosomatids, Hemiptera, Phytophagous insects, biocontrol.

VE39 - Interaction of *Blastocrithidia culicis* with midgut and salivary glands of *Aedes aegypti*.

Nascimento, MTC *(UFRJ)*; Arongaus, AB *(UFRJ)*; Dias, FA *(UFRJ)*; Lopes, AH *(UFRJ)*; Motta, MCM *(UFRJ)*; Saraiva, EM *(UFRJ)*

Blastocrithidia culicis is a monoxenic protozoan of the Trypanosomatidae family, which presents interesting characteristics such as evolutionary and biochemistry relationship with pathogenic trypanosomatids and easy axenic cultivation. Our group have recently shown that *B. culicis* was not only able to adhere to *Aedes aegypti* midgut but also, to live and multiply in this mosquito for at least 48 days. Here, we have further characterized the midgut receptor(s) responsible for the recognition of the protozoa. With this aim, we resolved total midgut proteins by SDS-PAGE followed by Western blot, identifying bands able to bind biotinylated-*B. culicis*. One protein with molecular weight of about 29 kDa which recognized stationary-phase *B. culicis* was identified in the total extract of *A. aegypti* female midgut. This protein was not observed in the the insect midgut protein profile which were naturally biotinylated. Previous results from our group demonstrated that *B. culicis* is able to reach the mosquito haemocele, suggesting the possibility of protozoa interaction with salivary glands; similar to what happens in malaria. To address this question we incubated excised salivary glands of *A. aegypti* females with *B. culicis in vitro*. Our results demonstrated that protozoan parasites at logarithmic phase culture bound better to the salivary glands than parasites at stationary phase of growth. The interaction between the protozoan parasites and the insect glands were higher after one hour than after four hours, for parasites at stationary growth phase, whereas the time of interaction had no influence for parasites at logarithmic growth phase. Supported by: CAPES, CNPq, FAPERJ

VE40 - MORPHOLOGICAL CARACTERIZATION OF THE SENSORIAL ORGANS OF THE ANTENNAE OF THE FEMALES OF Lutzomyia ovallesi (DIPTERA: PSYCHODIDAE), VECTOR OF CUTANEOUS LEISHMANIASIS IN VENEZUELA.

Pinto L.C. *(CPqRR/FIOCRUZ)*; Bahia-Nascimento A.C. *(CPqRR/FIOCRUZ)*; Secundino N.F.C *(CPqRR/FIOCRUZ)*; Pimenta, P.F.P *(CPqRR/FIOCRUZ)*; Fernandes, F.F. *(UFGO)*; Nieves, E. *(LAPEX)*

Lutzomyia ovallesi is a species responsible for the transmission of cutaneous leishmaniasis in Venezuela. The sensilla, sensorial organ of insects, are responsible for the recognizing stimulus and are possibly involved in the feeding process, as well as, aggregation, mating, noticing odors, humidity and temperature. The sensilla located in the antennae are important organs of reception of stimulus from the environment. This study analyzes the ultra-structural morphology of the sensorial organs of the antennae of the female using the Scanning Electron Microscope. Our results showed that this specie has a pair of thin antennae composed of sixteen segments. It was possible to identify the presence of six subtypes of sensilla classified as: squame, smaller trichoid, blunt-tip trichoid, coeloconic, chaetic and pointed tip trichoid. The first segment, scape, has a triangular shape and it was possible to observe the presence of blunt-tip trichoid sensilla. The pedicel has a spherical shape and the following types of

sensilla squame, smaller trichoid and blunt - tip trichoid. It was possible to visualize fourteen extremely thin flagellomeres with a smooth surface posterior to the pedicel. The first flagellomer is different from the others due to its superior size. No significant changes were noticeable from the second to the twelfth flegellomeres. In all antenna segments, it was observed the blunt-tip trichoid, pointed tip sensilla and spinules with no innervations, known as microtrichia. The twelfth, thirteenth and fourteenth flagellomeres were morphologically different from remained segments due to its more globular format, presence of a wrinkled type surface, and also, the presence of the coeloconic sensilla. Our observation can allow future taxonomic and filogenetic studies with the objective of clarifying evolutionary aspects of specified species of arthropods. Supported by: FAPEMIG

VE41 - *Aedes* immune responses to virus infection

Nascimento-Silva, M.C.L. *(UFRJ)*; Oliveira, L.S. *(UFRJ)*; Oliveira, P.L. *(UFRJ)*; Sorgine, M.H.F. *(UFRJ)*

Diseases caused by arthropod-born virus are significant public health problems, and novel control methods are needed to block pathogen transmission. Among them, one of the primary concerns is Dengue virus (*Flaviviridae*), which causes dengue fever and dengue hemorrhagic fever in tropical and subtropical areas. Although *Aedes aegypti* is the major vector of both yellow fever and dengue virus, very little is known about how the mosquito responses to virus infection. The understanding of insect immunity has undergone great progress in the last years since it has been shown that insect innate responses are very similar to vertebrate ones. Despite the enormous importance of understanding the innate immune system of disease vectors, little is known about mosquitoes immunity pathways. In this work we used TIGR database to select two hundred immune related genes that appear to be differentially expressed upon dengue virus infected *A. aegypti* EST libraries when compared with other *A. aegypti* EST libraries. The selected genes were divided in the following groups: Caspases, IAPs, serpins, TEPs, AMPs, Imd pathway, TLRs pathway The genes were amplified by PCR using T3 and T7 vector primers and are currently being spotted onto a macroarray chip in UNESP. mRNA of C6/36, CCL-125 cells and *A. aegypti* mosquitoes, Dengue infect or not, will be used as probes in order to identify significant chances in the expression of selected genes. This approach will give us a better understanding of how the mosquito immune system generally respond to virus infection, allowing more specific investigations of each immune pathway and of the mediators involved in this process.

VE42 - Parasitoidism of triatomine bugs (Hemiptera:Reduviidae) by *Megaselia scalaris* (Loew) (Diptera:Phoridae).

Jane Costa *(IOC/ FIOCRUZ)*; Gleidson Magno Esperança *(IOC/ FIOCRUZ)*; Carlos Eduardo Almeida *(IOC/ FIOCRUZ)*; Jacenir Reis dos Santos-Mallet *(IOC/ FIOCRUZ)*; Teresa Cristina Montes Gonçalves *(IOC/ FIOCRUZ)*; Angelo Pires do Prado *(UNICAMP)*

Megaselia scalaris, known as scuttle fly, is a cosmopolitan and synanthropic fly very eclectic in its habits acting as parasite, facultative parasite, parasitoid, and presenting several distinct feeding patterns. Here we report the first record of parasitoidism of triatomine bugs by *M. scalaris*. Specimens of *M. scalaris* invaded the containers in which the colonies of *Triatoma brasiliensis* were being reared under laboratory conditions. Two weeks later, it was possible to observe triatomines in atypical darker color with stretched out proboscis and legs, presenting difficulty to move at the bottom of the containers. All the developmental stages of *M. scalaris* were found inside the containers of the triatomine colonies. The parasitoidism was then confirmed by dissecting the symptomatic triatomines. Two fifth instar nymphs and nine adults were internally observed. Two nymphs and four adults had the internal organs so destroyed that no viscera could be found. Two adults had semi-destroyed viscera. In those ones, only a small portion of the digestive system could be found at the post mesenteric part, where pupae were found along the intestinal tube of both specimens. Another adult presented partial damages at the promesenteric portion, trachea, Malphigian tubules and rectal ampoule. Two pupae were found between the thoracic and abdominal portion where it was supposed to be seen the connection between the esophagus and promesenteries. The last two examined specimens presented only part of the digestive system destroyed, and no pupae were found though. It is important to mention that all specimens had their thoracic muscles entirely destroyed even the specimens presenting viscera remains. This finding corroborates the supposition that insects infested by *M. scalaris* present difficulty to move due to the progressive loss of muscle tone. Financial support: CNPq, FAPERJ, CAPES.

VE43 - Study of the pathogenicity of *Trypanosoma rangeli* in *Rhodnius prolixus* (Heteroptera: Triatominae: Reduvidae)

Kosmann, C. *(UFSC)*; Oliveira, T.L.B. *(UFSC)*; Serpa, V. *(UFSC)*; Caralho Pinto, C.J. *(UFSC)*

Triatomine are vectors of *Trypanosoma rangeli*, wich is the the second most frequently specie of genus *Trypanosoma* find infecting humans in Latin America. Despite its non-pathogenic characteristics for vertebrate hosts, its sympatric with *T. cruzi* producing crossing infections in the invertebrate host. Consequently, it generates crossed reactions in serological assays in the vertebrate host and that makes diffi-

cult the specific diagnosis of Chagas disease. The aim of this study was to verify the pathogenicity of *T. rangeli* on biological cycle and the reproductive aspects of *Rhodnius* prolixus. Recently emerged nymphs were grouped in a control group of 50 nymphs and in another four groups of 60 nymphs each. Of these, the first group was fed for 2,5 minutes and the others for 5, 7,5 and 10 minutes in artificial feeder with infected blood of *T. rangeli* (choachi strain) . The nymphs mortality was significantly different among the groups, despite no pattern was observed. The duration of the first instars was higher in all infected groups when compared with the control. The group fed for 10 minutes presented a lower oviposition rate that the control group. The same group also showed a hatching rate significantly different in relation to the group fed for 7,5 minutes. Deformations were found in all groups, being directly proportional to the infection time. These results suggest that the parasite draws out the time that nymphs remain on the firsts instars, without increase their mortality, as well as it diminishes the reproductive performance in accordance with the increase of the parasitic load. The high number of deformations observed suggest that *T.rangeli* exerts a great influence on the emergence and these deformations is influenced by the number of ingested parasites.

VE44 - STUDY OF THE MORPHOLOGICAL CHANGES IN THE OVARIAN DEVELOPMENT OF *Culex quinquefascitus*, BY ELECTRON SCANNING MICROSCOPE AND LASER SCANNING MICROSCOPE

Nacif Pimenta, R *(CPqRR-Fiocruz-MG)*; Botelho, A.C *(CPqRR-Fiocruz-MG)*; Kessen, F. F *(CPqRR-Fiocruz-MG)*; Pimenta, P.F.P *(CPqRR-Fiocruz-MG)*; Secundino, N.F.C *(CPqRR-Fiocruz-MG)*

Culex quinquefasciatus is a mosquito responsible for the transmission of human lymphatic filariasis. In this study different morphological techniques were used to analyze the change that occurs to the ovaries before, during and after the blood meal. Scanning Electronic Microscope (SEM) allowed the analyses of the topographical and morphological aspects of the ovary. When comparing the measurement of the different times after blood meal using SEM, it is interesting to note how much the ovaries increase from the time before the blood meal and after. During the blood meal the ovaries grow immensely in size, a single follicle from a female 72h after blood meal is bigger than a whole ovary of an unfed female. The ovarian membrane that surrounds the ovary was observed and this structure changes from a very rough aspect, at the beginning ovarian development, to a very thin with fewer numbers of filaments at 72h. The chorionic patterns were also visible at 72h after blood meal. The Laser Scanning Microscope allowed a view of the interior of the ovary. It was possible to see all of the structures that makeup a follicle. The nurse cells, the oocyte and the follicular epithelium were visible during the different times after blood meal. When comparing unfed ovaries with ovaries that have already laid the eggs, it is possible to note a slight disorganization of the ovarian membrane as well as an increase in the amount tracheas inserted in the ovary. The biochemical analyzes of the ovary confirms the continuous protein uptake that occurs. Even when there were no visible changes in the first hours after blood meal when looking through the SEM and LSM, the biochemical analyzes shows the absorption of proteins. This study is important to better understand the mechanic morphology of the ovaries during the development.

VE45 - Influence of the temperature, larval density and diet in the life cycle of Anopheles aquasalis

Carlos B. C. *(UNESP)*; Machado F. P. *(UNESP)*; Cruz E. C. F. L. *(UNESP)*; Rodrigues L. M. S. *(UNESP)*; Neto J. A. S. *(UNESP)*; Ribolla P. E. M. *(UNESP)*

The Culicidae Family, Diptera Order, possess 500 species in Brazil, 20 of which are of medical importance, including: *Anopheles darlingi*, *Anopheles aquasalis*, *Anopheles albitarsis*, *Aedes aegypti*, and *Culex quinquefasciatus*. Only females present blood feeding, important for the maturation of eggs, being also necessary the sugar intake, that constitutes the only food source for the adult males and power source for the flight of both sexes. Three variables had been tested in this experiment: the temperature and the density on development of the *A. aquasalis* life cycle, and the importance of the diet of carbohydrates on the physiological processes related to the longevity and fecundity of the same ones. To evaluate the temperature and density, we utilized 6 trays, 3 of them counting 100 larvae (L1) and the other 3 containing 300 larvae. One pair of tray (100 and 300) was undergone to 25^{o}C, other pair to 28^{o}C during the whole cycle. The last pair was kept at 28^{o}C until pupa stage, which were transferred to 25^{o}C until the egg laing. It was verified that the major larval mortality factor was the density. To analyze the carbohydrate diet, 360 mosquitoes were separated in cages feed with: sucrose and blood; water and blood; only sucrose. The adult mortality, eggs laing and larval formation were evaluated daily. The group A had the lowest mortality. The group B had high mortality mainly after the blood feeding, and the lowest eggs laing rate and larval formation when compared with the control group. The sucrose was showed essential to mosquitoes survival and eggs viability. Therefore, it is supposed that exists a possible relationship between carbohydrate ingestion and peritrophic matrix and/or spermatozoid maturation.

VE46 - HEMOCYTES FROM UNINFECTED TRIATOMINES INTERACT WITH SERA FROM CHRONIC CHAGASIC PATIENTS, BUT NOT AFTER THEIR SUCCESSFUL TREATEMENT

LEVY AMA *(IDPC)*; NAKAMURA PM *(IAL)*; LOURENÇO AMA *(IDPC)*; HOSHINO-SHIMIZU S *(FCFUSP)*; NUNES E V *(IAL)*; TANIGUCHI, HH *(IAL)*; FRAGATA FILHO AA *(IDPC)*

Immunological relationship between hemocytes from uninfected hematophagous vector species of *Trypanosoma cruzi* and infected mammal hosts were investigated. We have previously observed that sera from chagasic patients (Chs) recognized hemocytes from normal triatomines *(Triatoma infestans, T. palidipennis, Dipetalogaster maximus, Rhodnius prolixus and Panstrongylus megistus)*, but not hemocytes from a phytophagous bug. Here, this recognition was demonstrated to differ with sera from successfully treated chagasic patients (ChTs). Immunoblotting technique of hemocytes from *P. megistus* performed in sera from 26 chronic chagasic patients, before and after the chemotherapy, showed that Chs were always reactive. However, ChTs from same patients exhibited varied results, although these Chts were always negative for the *in situ* indirect immunofluorescence, a reaction which detects ongoing parasitemia. A previous absorption of Chs with formaldehyde fixed cultured epimastigotas, as well as, trypomastigotes from celular culture revealed that a substantial number of bands diminished after the absorption with latter parasites, but not with the absorption with former parasites. Proteins of 61-70, 41-50, 31-40 and 21-30 kDa were the major proteins involved in the process of serum absorptions. After chemotherapy, protein bands of 121-130, 91-100, 81-90 and 51-60 kDa disappeared in unabsorbed sera. Thus, hemocytes and trypomastigotes share common epitopes, and these epitopes are potentially useful for immunodiagnosis, immunoprotection, and evaluation of chemotherapy in Chagas' disease control.

VE47 - Natural Infection of Phlebotominae Sandflies in Corinto Municipality - Minas Gerais/Brazil

SARAIVA, L. *(CPqRR)*; QUARESMA, P.F. *(CPqRR)*; LIMA, A. C. V. M. R. *(CPqRR)*; FERREIRA, E.C. *(CPqRR)*; CARVALHO, G.M.L. *(CPqRR)*; VITOR, H.H.S. *(CPqRR)*; FALCÃO, A.L. *(CPqRR)*; ANDRADE FILHO, J. D. *(CPqRR)*

The survey of sandflies on the border of Lassance and Corinto municipality, in Minas Gerais Brazil, was carried out from July, 2003 to June, 2004. Systematic catches of sandflies were accomplished, using Falcão light traps in both country districts. Meanwhile, other catches were carried out using Shannon traps in rural locality in Corinto, aiming at determining the infection rate of species captured, and identify the species of *Leishmania* that occur there. Monthly catches were carried out in two consecutive days inside the hen house in the area, totalled 24 catches. The trap has worked, at least, for four hours a day from 6:00 P.M. to 10:00 P.M. All sandflies were put in plastic pots and were fed with sugar solution. Live females were dissected, identified and examined for flagellates. Saline suspensions of promastigotes from positive sandflies were inoculated into the hind feet of hamsters. DNA was extracted from their samples of liver, spleen and skin. This DNA was amplified through Polimerase Chain Reaction (PCR), using specific primers to the conservation region mini-circle kinetoplast. Amplified products were analyzed trough electrophoresis on agarose gels at 1.5% stained by ethidium bromid. Male and female sandflies non-dissected, were put in alcohol at 70% for further identification. We have dissected 386 females and we have found flagellate in two of them: one belongs to *Lutzomyia (Nyssomyia) neivai* (Lutz & Neiva, 1912) and the other to *Lutzomyia sallesi* (Galvão & Coutinho, 1939). The infection was peripylarian in the first female and suprapylarian in the second one. PCR using primers for *Leishmania* genus was positive for both skin samples. Natural infection rate was 0.54%. Further specific reactions of these samples will be carried out for correct identification of the infecting *Leishmania* in both phlebotomine species.

VE48 - EXPERIMENTAL INFECTION OF COLONIZED Aedes aegypti WITH DENGUE VIRUS 2

CAMPANELLI, E.S. *(CPqRR - FIOCRUZ/MG)*; BOTELHO, A.C.C. *(CPqRR - FIOCRUZ/MG)*; MARTINS, J.S. *(Funed-MG)*; SOUZA, K.P.R. *(Funed-MG)*; SECUNDINO, N.F.C. *(CPqRR - FIOCRUZ/MG)*; CECÍLIO, A.B. *(Funed-MG)*; PIMENTA, P.F.P *(CPqRR - FIOCRUZ/MG)*

The dengue viruses are a threat to more than 2.5 billion people, who live in endemic areas. This mosquito-borne disease is a major public health problem. *Aedes aegypti* is the primary vector of dengue in the Americas and is considered the most tractable mosquito species for laboratory culture, being used for detailed laboratory investigations of mosquito biology such as morphology, physiology and vector competence. The present study shows the susceptibility of a colony strain of *Ae. aegypti* to the Dengue virus 2. Two-hundred *Ae. aegypti* females (five-day-old) from a laboratory colony free from dengue infection were allowed to feed throughout a chick-skin membrane during 1 h in an artificial feeding device containing an infected blood meal constituted of heat-inactivated heparinized mouse blood mixed with virus-infected C6/36 cells (2:1). The engorged females were kept in small cages at 28°C and dissected in different days (3, 6, 9, 12 and 15 d). The midguts, ovaries, carcasses and heads were separated. The samples were fixed and immunolabeled with antibody anti-dengue 2 to be analyzed by laser confocal microscopy (LCM). Infected mosquitoes from the 15th day had their heads and bodies dissected, separated and frozen at -70°C to be analyzed by RT-PCR with specific primers. Immunolabeling of the midguts and ovaries showed the pres-

ence of Dengue virus 2 on the mosquitoes dissected on day 3 until the last day of the experiment. The RT-PCR reactions of the heads and the bodies confirmed the mosquito infection with Dengue virus 2. These results showed that the methodology used for the artificial infection with dengue virus was successful and demonstrated that our colonized *Ae. aegypti* mosquitoes are susceptible to artificial dengue infection. Future studies are in process to better understanding the invasion of the *Aedes* mosquito by the dengue virus.

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