
VT-1 – INSECT PATHOGENICITY OF A TRYPANOSOMATID ISOLATED FROM *PIEZODORUS GUILDINI*

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A Trypanosomatid (563 TD) was isolated from digestive tract of phytophagous Hemipteran *Piezodorus guildini* and cultivated in GYPMI medium at 25°C. This trypanosomatid showed to be fatal to phytophagous hemipterans.

An experimental infection was conducted on trypanosomatid non infected (“clean”) phytophagous hemiptera *Veneza zonata* (Hemiptera, Coreidae), raised from collected eggs and the nymphs feed on clean sherry tomatoes obtained from seeds at laboratory. The infection was done via haemocelle (10² culture cells) or on experimentally infected tomatoes.

Via haemocelle, the cells of haemolymph were apparently normal, most of them plasmatocytes, with no differences between 0 and 3 hours of infection. Starting with 7 hours, it was an increase of the population of prohaemocytes, with cells dividing and the appearance of bacteria. The enhancement of bacterial population was progressive at about 19 hours, been observed also a budding phenomenon of haemocytes. All the haemocelle-infected insects die about 24 hours after infection.

In the infection via tomatoes, the protozoa were observed at digestive tract about 6 days of infection and at haemolymph about 9 days after infection. It was not observed salivary glands infection and die occurred in all insects between 12 and 14 days.

Externally there was no signals of the infection and the strain 563 TD, belonging to genus *Leptomonas*, was highly pathogenic to *Veneza zonata*, important vector of trypanosomatids of plants, probably by lowering insect defenses against bacterial infection.

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VT-2 – SELECTION OF AN *Aedes fluviatilis* POPULATION TREATED WITH *Bacillus thuringiensis* AND THE RELATIONSHIP WITH *Plasmodium gallinaceum* INFECTION

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The culicidae family has crucial medical importance because of the transmission of several pathogens to man including that causing malaria. *Aedes fluviatilis* infected with *Plasmodium gallinaceum* has been used as a model to study the interaction between the malarial parasites and mosquito vectors. The use of *Bacillus thuringiensis* (Bti) as a larvicide is receiving increasing attention because of the appearance of resistance to conventional insecticides as well environmental problems caused by use of the latter. However, recent studies have also suggested some resistance of larva mosquitoes to Bti. In order to check this possibility and to know how Bti could affect the malaria infection rate of the mosquito vector in treated areas, we tested the vectorial ability of a population of *Ae. fluviatilis* selected for resistance in laboratory conditions. We used bioassays in which LC₅₀ was calculated to select survival of mosquitoes during 9 generations. After the 3rd generation they already have developed 6 fold resistance. We dissected the midguts from larvae and adults and processed them for SDS-PAGE to know the effects of Bti resistance in the mosquito. Preliminary results demonstrated characteristic protein bands present only in the guts of the Bti-treated mosquitoes. This finding raised the possibility of a different interaction process of the Bti-treated mosquitoes with pathogens. To test this hypothesis, we fed the two groups of mosquitoes directly on *P. gallinaceum*-infected chickens. Seven days after the infected bloodmeal, the midguts were dissected and the percentage of infected mosquitoes and the number of oocysts compared with the controls. The statistical analysis of three experiments showed no significant differences among the control and the Bti-treated groups (P>0.10) and we conclude that the effect of the biological insecticide in the mosquito vector does not alter the malaria infection rate.

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VT-3 – RHODNIUS PROLIXUS PROTEASE INDUCED BY TRYPANOSOMA CRUZI INFECTION

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Rhodnius prolixus is an insect that has been used as a model in studies of parasite-vector interactions by our laboratory. The interaction protozoan parasites with vectors may be modulated by a complex reactions. Several trypanosomes contain proteases that may be released into their invertebrate hosts. Previously published data demonstrated metalloproteases activities in the haemolymph of *R. prolixus* infected with *Enterobacter cloacae* (Feder et al., 1998) and *T. rangeli* (Feder et al., in press). Based in these facts, we decided to investigate proteases of *R. prolixus* infected with *T. cruzi*.

Using SDS-polyacrylamida gel electrophoresis containing gelatin as substrate, analysis of zimograms performed on samples of different tissues of controls and insects inoculated or orally infected. We observed protease activities in the haemolymph and fat body in a bloodsucking insect, *R. prolixus*, infected with *T. cruzi* (Dm28c). These proteases demonstrated distinct patterns of activities: (i) proteases were detected in the haemolymph of insects which were fed on, or inoculated with *T. cruzi* (Dm28c), but they were not observed in fat body of insects; (ii) protease was also presented in the fat bodies derived from naive insects or controls inoculated with sterile phosphate-saline buffer (49 KDa), as previously described with other microorganisms but it was not detected in the haemolymph of these insects. These proteases from the insect with *T. cruzi* will be characterized. The significance of these proteases will be discussed in relation to the success of the establishment of infection of these parasites in vector, *R. prolixus*.

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VT-4 – PROTEIN PHOSPHORYLATION DURING SALIVARY GLAND GROWTH IN THE BLOOD-SUCKING INSECT RHODNIUS PROLIXUS

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Rhodnius prolixus salivary glands contain a number of different anti hemostatic components such as antifactor VIII ; an antiplatelet activity; an apyrase activity; an antithromboxane activity and finally a vasodilator activity with the general properties of nitric oxide (NO). Although the mechanisms which regulate the production of those molecules are unknown. Protein phosphorylation is by far the most powerful mechanisms which control cellular activities. The presence of protein kinase activity in this system was studied using ^{32}P -ATP and dephosphorylated casein as substrate. When salivary glands from blood fed 4st nymphs were assayed for protein kinase activity this assay revealed a ten fold increase in enzyme activity 07 days after feeding. This enzyme activity (1.5 pmol ^{32}P incp./min/gland pair \pm 0.2) is kept activated on the first half of the feeding cycle. After moulting this activity decreases more than two fold (0.5 pmol ^{32}P incp./min/gland pair \pm 0.05). Endogenous substrates of this enzyme include a major phosphorylated 30 kDa protein as determined by SDS-PAGE followed by autoradiography. This protein is phosphorylated *in vitro* only after moulting when the protein kinase activity is smaller. Salivary gland extracts from days 07 and 30 after blood feeding were then fractionated on a Superose 6HR gel filtration column. Fractions were assayed for protein kinase activity using casein as substrate. These experiments revealed a single peak of protein kinase activity in both days with a molecular weight around 130 kDa. The inhibition of enzyme activity after moulting is also verified using this material. We further analyzed apyrase activity during blood feeding cycle. This activity is three fold increased after moulting (30 nmol Pi released/min/gland pair \pm 5.0 on the first half of the feeding cycle to 90 nmol Pi released/min/gland pair \pm 5.0). When apyrase activity was analyzed on salivary gland extracts from days 07 and 30 after fractionation on Superose 6 Hr the increase in apyrase content is also found after moulting. Together these data suggest some relationship between protein phosphorylation events and the level and/or apyrase content in *Rhodnius* salivary glands.

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VT-5 – ULTRASTRUCTURAL AND CYTOCHEMICAL ANALYSIS OF HEMOCYTES FROM *RHODNIUS PROLIXUS* (HEMIPTERA:REDUVIIDAE) INFECTED WITH *TRYPANOSOMA RANGELI* (TEJERA, 1920).

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Trypanosoma rangeli is an hemoflagellate protozoan that infects domestic and wild mammals, including man. Its distribution comprises Central and South America. The invertebrate host is the triatomine *R. prolixus*, the same vector of *T. cruzi*, the causative agent of Chagas' disease. It is not uncommon to find *R. prolixus* infected with both parasites. The life cycle of *T. rangeli* in invertebrate host begins in the intestine, where the parasites multiply and pass across the epithelial cell by an intracellular route, arriving into the hemocel. In this phase, the parasites are able to multiply freely in the hemolymph or invade hemocytes, where they apparently are able to multiply. The aim of this study is to determine which hemocytes are involved in cellular immune response against *T. rangeli* and verify if the parasite is able to multiply inside hemocytes. The insects were infected by an intra-celomic inoculation of 10^4 parasites and after 24 hours the hemolymph was collected daily. For scanning electron microscopy, the hemocytes were adhered to coverslips with 6 mm diameter and fixed in 2,5% of glutaraldehyde, 4% of paraformaldehyde diluted in 0,1M cacodylate buffer, pH 7,2. After 2 hours cells were washed 3 times with the same buffer, post-fixed in 1% of osmium tetroxide and 0,8% of potassium ferricyanide for 1 hour, washed, dehydrated, critical point dried and covered with a 20nm layer of gold and observed in a scanning electron microscope. For transmission electron microscopy, the hemocytes were fixed as described above, dehydrated and embedded in Epon. 60-80nm thick sections were stained in uranyl acetate and lead citrate and observed in the transmission electron microscope. In addition we utilized two cytochemical techniques to analyze alterations of the hemocytes. Thiery technique and acid phosphatase detection were performed as described in the current literature. The results showed that parasites interact first with granular cells and plasmotocytes, and just the last type of hemocytes present parasites in its interior, confined inside a vacuole. In the interior of the plasmotocytes the parasite could present a normal or a degenerated aspect. It was common to find plasmotocytes presenting more than one parasite in the cytoplasm, always in different vacuoles. Thiery technique showed that inside vacuoles there is a significant amount of polysaccharides when the parasites was well preserved. The acid phosphatase detection showed that when the parasites are intact the vacuoles do not show a positive reaction for acid phosphatase. Until now it was not possible to observe parasites inside a vacuole presenting a positive reaction. We can suggest that just some parasites are able to multiply inside plasmotocytes, whereas others are killed. The data obtained by Thiery technique and acid phosphatase detection suggest that parasites inside hemocytes are able to modify or escape from microbicidal mechanisms.

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VT-6 – INTERACTION OF *RHODNIUS PROLIXUS* LIPOPHORIN WITH ITS BINDING SITE AT THE FAT BODY

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Lipophorin is the major hemolymphatic lipoprotein in insects, and it is known to be involved in the transport of lipids between different organs in the hemolymph of *Rhodnius prolixus*. In this insect lipophorin transfers phospholipids to the oocytes, midgut and fat body and can be reloaded with more lipids and reutilized. For delivering or taking up lipids, lipophorin interacts with tissues by means of specific binding sites at cell surface, and we are studying the lipophorin receptor on *Rhodnius prolixus* fat body.

Fat body membrane preparation was obtained from adult females four or five days after blood meal. Purified lipophorin was radiolabelled in protein moiety (^{125}I -LP) and incubated with membrane preparation in the presence of bovine serum albumin and calcium, for the receptor characterization.

In the presence of increasing concentrations of membrane protein, corresponding increases in lipophorin (^{125}I -LP) binding were observed. The specific binding of lipophorin to the membrane preparation was a saturable process, with a K_d of $2.1 \pm 0.4 \times 10^{-7}$ M. Lipophorin binding did not depend on calcium and was strongly inhibited by an increased in the ionic strength. Suramin, a polysulfated polycyclic hydrocarbon known to inhibit the binding of lipoprotein to their receptor, also interfered with lipophorin binding to the fat body receptor, and binding was completely abolished at a concentration of 2 mM suramin but at concentration of 0.05 and 0.1 mM it seemed to increase the binding activity. Lipophorin from *Manduca sexta* was also recognized by *R. prolixus* fat body receptor, as it competed with *Rhodnius* ^{125}I -LP for the binding to the membrane preparation. The study of this receptor properties is important for the understanding of the regulation of lipid transport in hematophagous insects.

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VT-7 – PRELIMINARY CHARACTERISATION OF THE SYLVATIC ECOTOPE OF *TRYPANOSOMA CRUZI* TRANSMISSION IN THE FEDERAL DISTRICT OF BRAZIL

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Sylvatic *Trypanosoma cruzi* transmission has not been proven in the Federal District of Brazil, nor is domestic transmission of *T. cruzi* documented. Sylvatic triatomine bug species and mammals previously reported to be reservoir hosts of *T. cruzi* are abundant in the *Biome Cerrado* of Central Brazil (particularly in gallery forests). The WHO recommends further research on the distribution of non-domiciliated triatomine populations and reservoirs in enzootic foci. In preliminary work in gallery forest located in Núcleo Rural Rio Preto, Planaltina/DF the following methods were used: a) trapping and examination of marsupials and rodents by xenodiagnosis (with 10 nymphs of *Rhodnius neglectus*) and by thick films; b) tracking of trapped mammals to their refuges by means of a spool and line tracking device (Miles, M.A.; Souza, A.A. & Povoá, M.M. 1981. *J. Zool.*, 195:331-347) followed by microhabitat dissection; and c) examination of the crowns of palm trees for the presence of triatomine bugs. Sixteen animals were trapped and examined (12 marsupials and 4 rodents). *Didelphis albiventris* was found naturally infected with *T. cruzi* principal zymodeme I, which is known to be associated with *Didelphis* and *Rhodnius*. No bugs have yet been retrieved from located nests of *D. albiventris* but 14% of "Buriti" palms (*Mauritia flexuosa*) within 250 m of human dwellings were found to be colonised by *R. neglectus* and *Psammolestes coreodes*. So far no triatomine bugs have been found infected with *T. cruzi* or *T. rangeli*. The source of infection to *Didelphis albiventris* and the extent of the enzootic focus, and the possible future risk to local inhabitants remains to be evaluated fully.

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VT-8 – *TRYPANOSOMA CRUZI* DEVELOPMENT IN *RHODNIUS PROLIXUS*: INFLUENCE OF ECDYSONE AND DIFFERENT DIETS

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Recently our laboratory demonstrated that (1) decapitation of *Rhodnius prolixus* drastically blocked the development of *Trypanosoma cruzi* (Dm28c) and (2) head transplantation or therapy with ecdysone reversed the *T. cruzi* infectivity in decapitated insects. In the present study we explore the effect of decapitation and ecdysone therapy in insects infected with *T. cruzi* fed on whole blood and plasma alone and quantified epimastigote and metacyclic trypomastigote forms in the stomach, intestine and rectum of the insect vector. In all experiments the insects were decapitated just after feeding and the infection was done by adding 1.6×10^4 /ml of diet and 6.1×10^5 /ml of *T. cruzi* forms of trypomastigotes and epimastigotes, respectively. At different intervals after the insects fed on infected whole blood or plasma alone, the stomach, intestine and rectum were removed and gently homogenized in 1 ml phosphate-buffered saline (pH 7.2). Different *T. cruzi* forms and the number of parasites were quantified in a Neubauer hemocytometer. We demonstrated that insect infected with *T. cruzi* and fed on whole blood if decapitated the total number of parasites drastically decreased until 15 after feeding in the entire gut. However, decapitated insect treated with ecdysone maintained the infection as high as the infection in control insects. The epimastigote infection also was reversed by the treatment of decapitated insects with ecdysone. However, the number of trypomastigotes significantly diminished in decapitated insects and in insects decapitated receiving ecdysone therapy. The infections in the stomach were similar in the different groups of insects but the intestinal compartment seems to be the responsible for the decrease of infection in the decapitated insects since the epimastigote infection was reversed by ecdysone treatment but the trypomastigote infection not. The parasite infection in the rectum followed the same pattern of the intestine. We also demonstrated that the *T. cruzi* infection in insects fed on plasma alone presented no significant difference if the insects were treated with ecdysone. It seems that the presence of hemoglobin in the food and the hormone ecdysone may act interfering in the establishment of the *T. cruzi* infection in the intestine of *R. prolixus*.

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VT-9 – RADIATION EFFECT (CS 137) IN RHODNIUS PROLIXUS/TRYPANOSOMA RANGELI INTER-ACTION

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Trypanosoma rangeli Tejera, 1920, after being ingested as tripomastigotes by its vector *Rhodnius prolixus*, multiplies as epimastigotes in the midgut and invade the hemocoel. The epimastigotes survive in the hemolymph and/or inside the hemocytes, migrate to complete their development in the salivary glands. The literature is very poor about the effects of chemical and physical treatments on the interaction vector - parasite. In this study we investigated the radiation effect in the development of *T. rangeli* (H14 and Choachi strains) in *R. prolixus*. We applied different radiation doses ranged 400 to 4000 rads per insect. Our results demonstrated that doses higher than 1200 rads increased drastically the mortality. Insects treated with 400 and 1200 rads had the parasite colonization in the hemocoel anticipated for 7 days non radiated, and presented long and short epimastigotes of *T. rangeli* at high concentration in the hemolymph if compared with control infected insects. Since total counting of hemocyte and nodule formation were the same for all groups treated or not, apparently, the radiation did not affect the cellular immune response. Preliminary results demonstrated ultrastructural alterations in the midgut epithelial cells, mainly in cytoplasmic organelles. Some cells presented a general disorganization while others presented just a morphological alteration of organelles. The ultrastructural alteration in the gut epithelium was directly correlated to the doses of radiation used. The significance of the modified cell's gut in radiated insects is discussed in relation to *T. rangeli* invasion in the hemolymph.

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VT-10 – BIOCHEMICAL ASPECTS OF HAEM POLYMERIZATION IN RHODNIUS PROLIXUS MIDGUT

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Free haem (Hm) is a powerful generator of reactive oxygen species that can damage many biomolecules. Moreover, Hm can associate with phospholipid bilayers and interferes with their physical integrity, leading to membrane disruption. Many organisms developed efficient defences against the deleterious effects of free Hm. A special situation is found in *Plasmodium* parasites that during its life-cycle digest up to 80% of host cell haemoglobin producing large amounts of its prosthetic group Hm. Part of the freed Hm is sequestered into a dark-brown crystalline polymer called malaria pigment or haemozoin (Hz). In a previous work we identified Hz, as a product of haemoglobin digestion, in the midgut of the blood-sucking bug *Rhodnius prolixus*. Here, we investigated the process of Hm polymerization in *R. prolixus*.

Firstly, the particulate fraction from midgut luminal content of plasma-fed insects was able to promote Hm polymerization *in vitro*. This fraction contains extracellular phospholipid bilayers known as perimicrovillar membranes (PMM) which ensheat the microvillar membranes of the epithelial midgut cells. Also, a great number of membrane patches and vesicles, derived from the PMM, are thrown for the lumen of the intestine. Previous incubation of this fraction at different temperatures showed that the Hm polymerase activity is heat-labile and is not associated to the *Rhodnius* actinomycete endosymbiont *Rhodococcus rhodnii*. Moreover, this activity is strongly dependent of pH, temperature and time on Hz synthesis. The factor responsible for Hm polymerization is detergent soluble since the Hm polymerase activity increased more than three times when Hz synthesis promoted by midgut particulate fraction was carried out in the presence of n-octyl- β -D-glucopyranoside. Taken together these results suggest that Hm polymerase activity in *R. prolixus* midgut is promoted by an insect-derived factor and seems to be associated with PMM.

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VT-11 – HAEM POLYMERIZATION INTO HAEMOZOIN IN *RHODNIUS PROLIXUS* REDUCES HAEM RELATED DELETERIOUS EFFECTS

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Blood-sucking insects use haemoglobin as the major food source and its digestion release large amounts of haem. Free haem is a powerful generator of reactive oxygen species that can damage a variety of biomolecules. Also, haem can associate with phospholipid bilayers, causing structural alterations on the membrane and leading to cell lysis. Several organisms developed mechanisms to avoid the deleterious effects of free haem. In *Plasmodium* parasites part of the free haem (Hm) is sequestered inside digestive vacuolum into an insoluble dark brown crystalline polymer named malaria pigment or haemozoin. In a previous work of our group haemozoin was isolated and identified in *Rhodnius prolixus* (rHz). Here we investigated the pro-oxidant activity of rHz by several methodological approaches.

Thiobarbituric acid reactive substances (TBARS) were produced in the presence of haem, but not with rHz using azolectin liposomes as substrate. This result is in accordance with our previous data when linolenic acid or 2-deoxyribose were used as substrates for TBARS assay. TBARS production occurred at both neutral and acid pH, indicating independence of the reduced oxidant activity of rHz on the pH. Incubation of 10 µM of rHz with a 0,5% suspension of rabbit red blood cells caused no damage to the cells, while the same concentration of Hm led to complete haemolysis. Oxygen consumption, measured by a Clark electrode, did not occurred significantly when azolectin liposomes were incubated with 10 µM of rHz. However the addition of 10 µM Hm caused an intense decrease of oxygen tension at the same experimental conditions. It is important to note that previous treatment of rHz with 0,1 N NaOH, a condition which depolymerize the pigment producing monomeric Hm, led to a similar behaviour as described to Hm in all the above mentioned protocols. These results point out to a reduced pro-oxidant activity of rHz, compared to the monomeric Hm. Our present results strength the hypothesis that haemozoin synthesis in the midgut of *R. prolixus* represents an efficient way to avoid haem toxicity.

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VT-12 – ECTO-ATPASE ACTIVITIES ON MALPIGHIAN TUBULES OF *RHODNIUS PROLIXUS*

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Chagas disease is an endemic disease caused by a protozoan disseminated in Brazil. The natural transmission occurs by the parasite excretion by the blood-feeding vector a reduviidae insect. *Rhodnius prolixus* is a hematophagous insect which ingests large volumes of blood during a single feeding session. The digestive and excretory system of this insect consist of anterior, medium and posterior midgut, the Malpighian tubules and the rectum (Phillips, JE 1981 *Am. J. Physiol.* 241: R241-R257). The Malpighian tubules are in number of four and structures that connect with the region between the medium and the posterior midgut. They are formed by two segments, superior and inferior, and are in permanent contact with the haemolymph. The inferior tubule was a blindend. The primary function of Malpighian tubules are the transepithelial transport, that results in secretion of ions and water excess (O'Donnell, MJ and Maddrell, SHP 1984 *J Exp Biol* 110:275-290). The Malpighian tubules can be compared to the vertebrate kidney.

Ecto-ATPases are transmembrane enzymes that catalyze the hydrolysis of extracellular ATP to ADP and inorganic phosphate. These ecto-enzymes are found in various tissues, as smooth and skeletal muscles, nervous tissue and organs as lung, liver and kidney in different organisms, including human. In this study we detected and partially characterized ecto-ATPase activities in *R. prolixus* Malpighian tubules.

Malpighian tubules were dissected from *R. prolixus* adult males, fasted for 5 weeks, and were collected in a cold isosmotic solution (100 mM NaCl, 8.6 mM KCl, 20mM Tris-Hepes (pH 7.0), 34 mM glucose, 2.9 mM alanine) with composition similar to the haemolymph (Maddrell, SHP 1969 *J Exp Biol* 51:71-97). In this saline it was possible to measure ecto-ATPase activities. The enzymatic activity was linear with time until at least one hour and with the number of tubules. The ecto-localization of this enzyme was comproved by its insensibility to common inhibitors, as vanadate, molibdate and fluoride. Moreover this activity was inhibited by suramine, an E-Type ATPase inhibitor and antagonist of P₂ receptor. The tubules incubated with 1mM EDTA showed Mg-independent ATPase activity (11.6 nmoles Pi x h⁻¹ x mg⁻¹). However, in the presence of 1mM MgCl₂ the ecto-ATPase activity was 8.2 nmoles Pi x h⁻¹ x mg⁻¹. These data are indicating that Malpighian tubules of *Rhodnius prolixus* probably possesses, at least, two Ecto-ATPase activities, a Mg-independent and a Mg-dependent activity.

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VT-13 – IDENTIFICATION AND CHARACTERIZATION OF AN ECTO-ATPASE PRESENT IN *RHODNIUS PROLIXUS* FAT BODY

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Ecto-ATPases, are glycoproteic that hydrolyze extracellular nucleoside tri and/or diphosphates are located on the cell surface, and are present in a great variety of cell types. These ecto-enzymes are found in various tissues, as smooth and skeletal muscles, nervous tissue and organs as kidney, lung and liver in different organisms, including humans.

In this study we detected and partially characterized ecto-ATPase activities in *Rhodnius prolixus* fat body. This organ is involved with different aspects of the insect metabolism, as carbohydrate and lipid storage, synthesis of many proteins, and can be compared to the vertebrate liver.

Fat bodies dissected from *Rhodnius prolixus* adult females were incubated in the presence of [$\gamma^{32}\text{P}$]-ATP, and ATPase activity was determined by measuring ATP hydrolysis, by liquid scintillation. Similarly to other ecto-ATPases, the *Rhodnius* fat body enzyme was stimulated by MgCl_2 , CaCl_2 , MnCl_2 , SrCl_2 and ZnCl_2 (5 mM). Two ecto-ATPase activities, a Mg dependent and a Mg independent, were identified and both activities increased linearly with time until 30 minutes. The effect of pH was examined in both activities and in the pH range from 6.0 to 8.0 the ATPase activities were not affected.

In order to discard the contribution of other ATPase activities to the ATP hydrolysis and to better characterize this ecto-enzyme, various specific inhibitors were tested. Both ATPase activities (Mg dependent and independent) were not inhibited by vanadate (1mM), NaF (1mM), sodium phosphate (5mM), pNPP (5mM) and tartrate (1mM), indicating that ATP hydrolysis was not promoted by phosphatases. The lack of response to ammonium molybdate (1mM) indicated that a 5' nucleotidase did not contribute to the ATP hydrolysis. The absence of mitochondrial ATPase participation was shown by the use of sodium azide (1mM) and oligomycin (1 $\mu\text{g}/\text{mL}$). Na^+/K^+ ATPase activity was not present as indicated by the use of ouabain (1mM). The Mg dependent activity was inhibited by ADP and 5' AMP, indicating the presence of an ATP diphosphohydrolase, whereas the MgCl_2 independent activity was not affected.

The external localization of ATPases here studied was confirmed by the utilization of 1mM 2',2'-diisothiocyanoethylbenzene disulfonic acid (DIDS), that inhibited both activities.

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VT-14 – *RHODNIUS PROLIXUS*' SALIVARY GLANDS PHOSPHOPROTEINS

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Blood sucking arthropods salivary glands can harbor several different human parasites. Although the mechanisms which control salivary gland biology are unknown. Protein phosphorylation is the most powerful control mechanism for the regulation of cellular activities. In order to verify the presence of phosphoproteins under regulation during *Rhodnius prolixus* salivary gland growth 4th instar nymphs were metabolic labelled with ^{32}P . Insects were then dissected in different days after blood feeding and salivary glands removed. When this material was analyzed through SDS-PAGE followed by autoradiography a single phosphorylated 30 kDa band was visualized. This phosphoprotein seems to be accumulated during salivary gland growth. Insects were fed with ^{32}P and dissected 30 days later. Salivary gland extracts were obtained and fractionated through chromatography on Superose 6 Hr gel filtration column. ^{32}P content of each fraction was analyzed through liquid scintillation counting. This experiment showed a major peak of ^{32}P associated to a 20 kDa fraction of the column. Fractions were then pooled and applied on a Mono Q 5/5 Hr ion exchange column. Under these conditions two different peaks showed up, the first one is eluted in the void of the column and the second one is eluted around 0.2 M NaCl. Metabolic labelled salivary glands phosphoproteins were also analyzed through chromatography on chromatofocusing using a Mono P column. Extracts from 50 ^{32}P metabolic labelled salivary glands obtained in Tris-acetate 75 mM pH 9.3 were applied on the column equilibrated with the same buffer. A pH gradient with polybuffer acetate pH 6.0 was used to elute the proteins and revealed two different peaks of radioactivity. The first peak is eluted in the void of the column and the second one is eluted in pH 9.0. Both fractions are recognized by monoclonal antibodies against phosphoserine indicating the presence of this modified amino acid in the molecule. Finally 30 day old salivary glands were *in vitro* cultured with ^{32}P . Under these conditions a major low molecular weight phosphoprotein can also be labelled in a time dependent manner. The results above point to the presence of a family of low molecular weight phosphoproteins in the salivary glands of the blood sucking insect *Rhodnius prolixus*. In the future the isolation of these molecules as well as the obtention of their NH_2 -terminal sequences will provide some information about the role they play in salivary gland biology.

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VT-15 – α -GLUCOSIDASE IS A BIOCHEMICAL MARKER OF PERIMICROVILLAR MEMBRANES IN THE KISSING BUG *RHODNIUS PROLIXUS* AND OTHER HEMIPTERANS

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The insects of the order Hemiptera are characterized by the absence of the peritrophic membrane, a chitinous-proteic structure that envelopes the food bolus in the majority of insects. But the microvillar membranes of the intestinal cells are not in direct contact with the food bolus in hemipterans, because of the existence of the perimicrovillar membranes, which ensheathed the microvilli from their bases up to their tips and extending further to the gut lumen. These membranes, in contrast with the peritrophic membranes, are truly lipo-proteins containing membranes.

It has been suggested that a α -glucosidase is a biochemical marker of these structures in the cotton seed sucker bug *Dysdercus peruvianus* (Heteroptera: Pyrrhocoridae) (Silva *et al.*, 1995). This is the only known example of a biochemical marker of these membranes. We were able to demonstrate that adults of the kissing bug *R. prolixus* has a major membrane bound α -glucosidase, which presents many properties similar to those found for the enzyme from *D. peruvianus*. By using *in gel* assays with a fluorogenic substrate after SDS-PAGE, we observed that the *R. prolixus* α -glucosidase showed an electrophoretic migration very similar to that of the *D. peruvianus* enzyme, indicating that both enzymes have a relative molecular weight of 60,000. The similarity between the α -glucosidase of *R. prolixus* and *D. peruvianus* was reinforced by the observation that a polyclonal antibody raised against the enzyme of the phytophagous bug recognized the kissing bug enzyme by using cryoultramicrotomy studies. Gold labeling was intensely detected in the perimicrovillar membranes of the intestinal cells of *R. prolixus*. The same antibody was also capable to recognize the α -glucosidase from a cicada and an aphid, suggesting that this enzyme is very widespread among the insects of the order Hemiptera. Our results point to the fact that the α -glucosidase and the perimicrovillar membranes are conserved among these insects, revealing them as potential targets to control of these economically important pests and disease vectors.

Reference: Silva *et al.* (1995) J. Insect Physiol. 41, 1093-1103.

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VT-16 – VITELLOGENIN PHOSPHORYLATION SITES AFTER ENDOCYTOSIS IN THE OOCYTES OF *RHODNIUS PROLIXUS*

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Vitellogenin is a phospholipoglycoprotein which is stored in the oocytes during oogenesis. Inside the oocytes of nonmammalian vertebrates, vitellogenin is the target of specific proteases whose activity gives rise to a set of smaller yolk polypeptides: lipovitellins 1 (120 kDa) and 2 (30 kDa) and phosvitins (28-35 kDa). Phosvitin is the most phosphorylated protein found in nature: more than half of its residues are serines, and most of them are phosphorylated. The high degree of phosphorylation allows these proteins to bind cations. These ions will be further used during embryo growth. In invertebrates, vitellogenins contain less phosphate and fewer serines than in vertebrates and their proteolytic processing pathway after endocytosis is absent. *Rhodnius* vitellogenin (460 kDa) is also removed from insect hemolymph during oocyte growth giving rise to vitellin. We decided then to search for phosphoserine rich stretches on vitellogenin which could be derived from some post endocytosis processing by oocyte protein kinases. These sites are phosphorylated for casein kinase II which recognizes specifically acidic phosphorylation sequences on its substrates. *Rhodnius* vitellogenin was purified through ultracentrifugation of hemolymph in a KBr gradient, followed by extensive dialysis against water. The material was then centrifuged and applied in a DEAE Toyo Pearl. Vitellogenin peak was then diluted and applied on a Mono Q 5/5 Hr in HPLC. This protocol allowed us to obtain highly purified vitellogenin free of other hemolymphatic proteins. A similar protocol was used for the purification of vitellin from chorionated oocytes. Both molecules were trypsin digested for 2 hours at pH 8.0 and this material reacted with monoclonal antibodies against phosphoserine. This treatment revealed a 80 kDa phosphorylated polypeptide which contains most of the phosphorylation sites of both molecules. This fragment is more phosphorylated on vitellin than in vitellogenin which indicates some kind of post endocytosis phosphorylation. In order to verify the presence of casein kinase II phosphorylation sites in both molecules this enzyme was also highly purified from *Rhodnius* oocytes. Phosphorylation reactions demonstrated that casein kinase II phosphorylate additional sites on vitellogenin which are absent or already phosphorylated on vitellin. We now intend to obtain internal sequences of the 80 kDa polypeptide in order to determine if those sequences share any similarity with phosphoserine clusters found on nonmammalian counterparts.

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VT-17 – EFFECTS OF TWO PROTEINS (30 KDA AND 45 KDA), ISOLATED FROM CHORIONATED OOCYTES OF *RHODNIUS PROLIXUS*, ON CARDIAC CELLS AND FUNGI

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The egg of *Rhodnius prolixus* is composed of yolk, which contain a large amount of phosphorylated proteins, sugar and lipids. The yolk is surrounded by the cell membrane and it is protected against mechanical damage, desiccation and microorganisms by the eggshell. Here we describe the purification and partial characterization of two proteins from the eggshell which show a inhibition of *Aspergillus niger* growth. One of these proteins reversibly inhibits calcium channels of cardiac cells and the other apparently associate with cell membrane making it leaky. The proteins were extracted from eggshell in 8M urea and applied on SDS-PAGE. The 30 kDa and 45 kDa proteins were recognized, cut out from the gel and removed from using ammonium bicarbonate.

The purified proteins were used to test its effect on the the *Aspergillus niger* growth. The growth of *Aspergillus niger* was monitored at 25°C during 96 h in 120 µl of culture medium (YNB) from Difco. Both proteins inhibited 100% of *Aspergillus* growth at the final concentration of 200 mg/ml.

Effects on calcium channel was evaluated in isolated cardiac cells using the whole cell configuration of patch clamp method.

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VT-18 – THE ROLE OF EICOSANOIDS IN THE ENDOCYTOSIS IN *RHODNIUS PROLIXUS* OOCYTES

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Oogenesis plays a central role on insect reproduction. During its development a wide range of proteins are accumulated by insect oocytes. Several of these proteins are removed from hemolymph by specific receptors in oocyte's surface. A heme binding protein was isolated from the hemolymph and eggs of the blood sucking bug *Rhodnius prolixus* and characterized (Oliveira et al. 1995, JBC 270, pp:10897-10901). Purified RHBP labelled with ¹²⁵I in protein moiety was then used in this study in order to investigate its endocytosis by the ovaries *in vivo* and *in vitro* (as characterized by Machado et al., 1998, Arch. Insect Biochem. Physiol. 39, pp133-143). ¹²⁵IRHBP endocytosis was studied *in vivo* through injections of protein in insect hemolymph, and the radioactivity incorporated by ovaries was estimated by gamma counting. *In vitro*, the ¹²⁵IRHBP incubated in the presence of ovaries was specifically removed from the media by selective receptors. These results show that *Rhodnius* oocytes have specific binding sites for RHBP at their surface. These approaches also permit to study endocytosis regulation in *Rhodnius* oocytes. In addition the participation of eicosanoids and second messengers in regulation of RHBP endocytosis was addressed. The rate of RHBP increased in the presence of indomethacin (a potent cyclooxygenase inhibitor), both *in vitro* (up to 50%) as *in vivo* (up to 20%). The serine/threonine protein phosphatase inhibitor, okadaic acid (1µM), decreased *in vitro* the RHBP uptake (up to 70%). Together these data show that RHBP endocytosis may be under protein phosphorylation control, and local mediators such as eicosanoids. Phosphoproteins involved in this process are now being identified in the ovaries by immunoblot with antiphosphoaminoacids antibodies.

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VT-19 – MORPHOLOGICAL STUDIES OF *RHODNIUS BRETHESI* MATTA, 1919 (HEMIPTERA, REDUVIIDAE, TRIATOMINAE) BY SCANNING ELECTRON MICROSCOPE AND CONFOCAL

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The aim of this study is to analyze the ultra-structure of the eggs exochorion, and cuticular structures of nymphs and adults of *R. brethesi* with taxonomic diagnosis. The specimens were provided by the bug colony of the Tropical Medicine Department, Oswaldo Cruz Foundation. The methodology was the same as the one described by Ferro et al., 1998 (Mem. Inst. Oswaldo. Cruz, RJ. Vol. 93, Suppl. II, Nov.). Micrographs were performed in scanning electron microscope and confocal. The eggs present a lateral flattening, characteristic of the genus *Rhodnius*. The operculum exochorion presents hexagonal cells and some pentagonal, both of smooth aspect (Mascarenhas, 1982, Amazon Acta 12 (3): 661-664). The body of the egg presents exochorion with granular hexagonal cells in flat funnels and one follicular pit per cell. In the chorial rim were visualized spermatid groove, micropylae and aeropylae (Barata, 1981, Rev. Public Health, 15: 490-542), as well as a circular micro-structure, that is under analysis phase. In the antennae, sensillas denominated trichobothrias located in the second antennal segment or pedicel, were studied (Catalá, 1997, Atlas of Chagas' Disease Vectors in the Americas I: 74 - 83). A trichobothria in the subapical area of the pedicel in all nymphal instars and at least nine in the adult specimens that are distributed from the base up to the apex of the pedicel was visualized, different from what is shown in Lent & Wigodziky, 1979 (Revision of the Triatominae (Hemiptera, Reduviidae) and their significance as vectors of Chagas' Disease. Bull. Am. Mus. Nat. Hist., 163 (3):146). The cuticular area, in the base of the trichobothria, presents regular grooves with thin, finger-shaped prolongation, different from the rest of the integument of the pedicel. The labrum is pear-shaped and it rests on the first segment of the rostrum that presents, laterally, a regular and symmetrical granulation. The integument of the labrum presents short and thick bristles, generally curved down. And, in the integument of the adult labrum, "star-like" structures were observed. The apex of the rostrum presents sensillas with different forms and sizes (Catalá, 1996, J. Morphology 228: 195 - 201). Stridulatory sulcus, where the apex of the rostrum rests, are formed by parallel bars. Two cuticular structures stand out in the apex of the tibiae: the ctenidium that is present in all nymphal instars and the spongy fossula present just in the adult specimens. So as to confirm some structures that are being analyzed, a greater number of *R. brethesi* will be observed.

VT-20 – THE INFLUENCE OF TEMPERATURE AND HUMIDITY ON THE LIFE CYCLE OF *RHODNIUS NEGLECTUS* LENT, 1954 IN LABORATORY (HEMIPTERA, REDUVIIDAE, TRIATOMINAE)

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Global warming and the possible physical and ecological impacts are waking up researchers to several areas of interest. The impact that climatic change can have on human health has been a theme of debate among specialists of WHO (WHO, 1996 *Climate change and human health*, Geneva, 298 pp.). In the case of Chagas' disease, the probable alterations in the biological dynamics of the insect vectors would be: modifications in the geographical distribution of the species; alterations in the dispersion mechanism of flight; increase in the number of bloodmeals, increasing the probability of transmission of the *Trypanosoma cruzi*; reduction in the duration of the life cycle and a increase in population density, causing a significant impact on the epidemiology of Chagas' disease (Burgos *et al.* 1994, *Entomol Vect* 1: 69-78; Carcavallo *et al.* 1998, *Entomol Vect* 5: 137-138). *Rhodnius neglectus* Lent, 1954 is distributed in States of Bahia, Goiás Mato Grosso, Minas Gerais and São Paulo (Carvalho & Verano 1956, *Rev. Goiana Med.* 2: 241; Barretto 1967, *Rev. Soc. Bras. Med. Trop.* 1:23 and Mello 1980, *Rev. Brasil Biol.* 40: 323-326). In spite of being a sylvatic species, it is frequently found in dwellings, according to Corrêa (1968, *Rev. Bras. Malariol. D. Trop.* 20:39-81) and Barretto *et al.* (1968, *Rev. Inst. Med. Trop. São Paulo* 27:145-156) it demonstrates a tendency to domesticity. The objective of this work was to establish the effect of different conditions of temperature and humidity on the development of *R. neglectus*. The insects used were from the colonies maintained at the Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos. 150 eggs were collected from the colony, all oviposited on the same day. These eggs were divided into 3 groups and maintained under the following conditions: a) 33 °C and 70% of R. U., b) 33 °C and 40% of R. U. and c) 28 °C and 70% of R.U. The insects were fed weekly on mice, and observed daily, for evaluation of the following parameters: period of egg development to adult, period of development of each instar, number of bloodmeals accomplished by instars and the percentage of mortality. The development period (in days) of each instar increased to the adult phase in the three observed groups, the shortest period of egg development to adulthood was observed in group b (81 days) and the longest in group a (150 days), demonstrating a greater influence of low humidity, when combined with a high temperature. With regard to the number of accomplished bloodmeals, there were no significant differences observed between the studied groups (Mann-Whitney P <0,01). The largest percentage of mortality was observed in groups a and b, demonstrating that, unlike the development period, the highest temperatures had a greater influence on mortality.

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VT-21 – POPULATIONAL DYNAMICS OF *RHODNIUS PALLESCENS* BARBER, 1932 UNDER LABORATORY CONDITIONS (HEMIPTERA, REDUVIIDAE, TRIATOMINAE)

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Rhodnius pallescens is considered to be the main vector of *Trypanosoma cruzi* in rural areas of Panama and is the only vector known of *T. rangeli* in this country. This species can be found both peridomestically and in sylvatic habitats in Belize, Colombia and Costa Rica. The objective of present work is to study the populational dynamics of a colony of *Rhodnius pallescens*, for a period of 24 months. The following observations were made: the period of eggs incubation, the percentage of ecdysis and mortality, the period of development of the eggs to adults and the age pattern of the colony recorded every 30 days. The aim being to try to establish correlations between seasonality, density and phases of development. Insects were taken randomly from the colony, maintained in the insectary of the Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos. Specimens of 5th instar nymphs to were coupled and placed in 3 glass container, maintained in ambient temperature, were in the observed period varied between 23.3 °C to 26.9 °C. Mice were offered for feeding every week for six hours. To this date only one couple has been. After 111 days of observation 136 eggs were obtained, of these 79 (58%) hatching. The percentage of mortality in first instar be considered decrease that compare to the other species of the genus. The percentage of mortality in first instar is 11.1% . 19.1% is the second instar nymphs, 8.8% in third instar and only one specimen in fourth instar. The longevity of the male was 104 days. This preliminary results have demonstrated a low biotic potential. It is important to point out that these observations were make in the winter , and a different pattern of results maybe obtained by running the experiment in the summer month under conditions of increased temperature.

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VT-22 – *TRYPANOSOMA CRUZI* TRANSMISSION CYCLE: SELECTION CONDITIONS IN THE SYLVATIC ENVIRONMENT

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Trypanosoma cruzi is transmitted by dozens *Reduviidae* species that may act as biological filters , influencing growth and/or metacyclogenesis rates. Also mammalian reservoirs can act as selective forces, maintaining or eliminating a given subpopulation of the parasite. Aiming to better comprehend the consequences of this interplay in the transmission cycle of *T.cruzi* in a given sylvatic environment we performed a study of the natural infection of marsupials (*Didelphis marsupialis* and *Philander opossum*) in Teresópolis, Rio de Janeiro State. The characterization of the isolates through biological parameters (mortality for swiss mice) we observed that the isolates of *T.cruzi* could be separated in two groups one, mainly associated with *P.opossum* and the other with *D.marsupialis*. The isolates derived from *Rhodnius prolixus* , the only triatomine species collected in the area were biologically similar to the *D. marsupialis* isolates. As we knew from experimental infections that *P.opossum* is able to maintain *D.marsupialis* subpopulations of *T.cruzi*, we suggested that two distinct transmission cycles were occurring sympatrically between these two phylogenetically related species. To confirm this hypothesis we followed up the experimental infection of *R. prolixus* with, respectively, one isolate from *P.opossum* and one from *D.marsupialis*. . We also compared the pattern of the experimental infection of bugs derived from our field specimens of *R. prolixus* , with insects from a long time laboratory maintained *R. prolixus* colony . Two groups of 50 bugs from each insect colony were allowed to feed through an artificial membrane on defibrinated rabbit blood containing 1×10^6 parasites/ml respectively, *D. marsupialis* and *P. opossum* strains (G-645 and PO13). Non engorged insects were eliminated and the remnants dissected 20, 30 60 days after the infecting meal. The whole intestine was grounded in PBS buffer and countings in Neubauer chamber were performed. It was observed that, in *R. prolixus* from the laboratory colony, both *T.cruzi* isolates developed and differentiated well and in a similar pattern. But the course of experimental infection of *R. prolixus* from the field colony differed significantly depending if *Philander* or *Didelphis* strain was used: infections with G-645 strain resulted in ten fold higher number of flagellates (epimastigotes and metacyclic forms) 20 and 30 days after the infecting meal. On the contrary, PO13 strain yielded 3x more parasites 60 days after the infection. We concluded: a) in Teresópolis, both *T. cruzi* isolates are well adapted to the insect vector therefore, *P.opossum* and *D. marsupialis* are participating from two distinct transmission cycles confirming our previous hypothesis on the complexity of the transmission cycle of *Trypanosoma cruzi* in the sylvatic ecotope. b) Not only the vector species but also its maintenance conditions influences the development of *T. cruzi*.

Supported by PAPES/FIOCRUZ.

VT-23 – HIGH LEVELS OF MITOCHONDRIAL DNA SEQUENCE DIVERGENCE AMONG *TRIATOMA BRASILIENSIS* NEIVA, 1911 POPULATIONS. (HEMIPTERA, REDUVIIDAE, TRIATOMINAE)

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Triatoma brasiliensis is the most important Chagas disease vector in the semiarid areas of northeast Brazil. The high degree of chromatic and morphological variation presented by this species has led, in the past, to its subdivision in three subspecies (*T. b. brasiliensis*, *T. b. macromelasoma* and *T. b. melanica*). Lent and Wygodzinsky (1979), however, invalidated these subspecific ranks stating that natural intermediate forms between them could be found. Recently, Costa et al. (1997a, b, 1998) and Costa and Marchon-Silva (1998) not only confirmed the distinctness of the three populations (subspecies) of *T. brasiliensis* based on morphological, isoenzymatical, ecological and biological data but also revealed the existence of a fourth undescribed population. In this study we compared by mitochondrial DNA sequence analysis, specimens belonging to the three proposed subspecies and also specimens from the recently reported chromatic population (juazeiro population). A 510 base pair region of the mitochondrial cytochrome B gene was sequenced from two specimens of each population (except for the melanica population where 4 individuals were analyzed). The sequences were aligned by eye and analyzed according to the Neighbor-Joining method, yielding a tree that reflected the following relationship: (melanica (juazeiro (brasiliensis, macromelasoma))). All pairs of specimens of each population analyzed presented the same haplotype. However, the pairwise sequence comparisons among the four populations revealed a large degree of sequence divergence, ranging from 2.7 (for the brasiliensis-macromelasoma pair) to 11.2% (for the brasiliensis-melanica pair). To assess the taxonomic meaning of such measures, pairs of closely related species, namely *T. infestans* / *T. platensis* and *T. sordida* / *T. garciabesi* were also analyzed. The percentages of sequence divergence obtained were 7.7 and 7.5%, respectively. These results show that the levels of sequence divergence found between some populations of *T. brasiliensis* is even higher than those found between other closely related triatomine species, and therefore strengthen previous observations suggesting that *T. brasiliensis* may hold more than a single biological species.

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VT-24 – COMPARATIVE STUDY OF INTERNAL ANATOMY AND MORPHOMETRY OF MALE REPRODUCTIVE SYSTEM OF TWO POPULATIONS OF *TRIATOMA BRASILIENSIS* NEIVA, 1911 (HEMIPTERA, REDUVIIDAE, TRIATOMINAE) – PRELIMINARY RESULTS

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Morphology of the genitalia has been largely used for taxonomic purposes in insects, however there are only few studies on the morphology of portions of the internal reproductive system of Reduviid bugs. In the present study we have performed a morphological analysis of the male reproductive system of *Triatoma brasiliensis* Neiva, 1911 (Hemiptera, Reduviidae, Triatominae), including a morphometry of the testicular follicles by both light and scanning electron microscopy. The specimens were obtained from colonies held in laboratory for 4 years, initiated with specimens caught in Caicó/RN (brasiliensis population), Espinosa/MG (melânica population), Petrolina/PE (macromelasoma population) and Juazeiro/BA (juazeiro population), Brazil. According to Costa (1997), these four populations can be distinguished by their morphological, biological, isoenzymatical and ecological aspects. The insects were fed weekly on Swiss albino mice and kept in a BOD incubator at 29°C and 80%RU with a 12 hours photoperiod. The dissection was done in a salt solution (0.7% NaCl 0.3% KCl) at the 3rd day after the imaginal ecdysis. The covering membrane of the testicular follicles was removed in order to allow their distention. Each follicle was drawn at a camera lucida and subsequently measurements were made from the drawings using a curvimeter along both side. Only the highest values were considered. Preliminary inter-specific comparisons, to long, medium and short testicular follicles were made through the analysis of variance between brasiliensis and juazeiro populations. The comparison have shown a significant difference ($P^3 0,001$) to the long follicles in the specimens obtained until now. In the medium and short follicles there were no significant difference. The others populations will be analyzed in the future.

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VT-25 – INFLUENCE OF BLOOD SOURCE ON *TRITOMA* BIOLOGY

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The objective of this study was to determine the influence of the blood meal source on the life cycle and reproductive development of females of *Triatoma infestans* (Klug, 1834), *Triatoma brasiliensis* Neiva, 1911, *Triatoma sordida* (Stal, 1859) and *Triatoma pseudomaculata* Corrêa and Espínola, 1964. The life cycle, mortality and fecundity of females were evaluate in insects fed on mice or pigeons. In all triatomine species studied, the life cycle was shorter for the groups fed on mice than for those fed on pigeons, the range of differences being between 1.5 times (*T. pseudomaculata* and *T. infestans*) and 2.4 times (*T. brasiliensis*). The values of total life cycle obtained on pigeons and mice were, respectively, 282.0 ± 13.2 and 119.7 ± 3.2 to *T. brasiliensis*, 247.0 ± 11.8 and 161.9 ± 4.1 to *T. infestans*, 335.4 ± 12.4 and 232.6 ± 9.7 to *T. pseudomaculata*, and 252.7 ± 18.4 and 119.7 ± 2.2 to *T. sordida*. The mortality rate of nymphs during the life cycle tended to be greater in insects fed on pigeons than those fed on mice, the differences for *T. brasiliensis* being statistically significant. The fecundity of females was different when the two feeding sources were compared, with the exception of *T. pseudomaculata*. For *T. brasiliensis* and *T. sordida* the higher mean of eggs/female were observed in females fed on mice, while for *T. infestans* these values were higher when females were fed on pigeons. Females of *T. sordida* and *T. pseudomaculata* had a greater fecundity than *T. infestans* and *T. brasiliensis* independently of the blood meal source. The differences of fecundity observed, probably reflect differences in the availability of blood in the natural ecotopes of these species, meals being more frequent for *T. infestans* and *T. brasiliensis*, which live at high densities in association with rodents in highly stable ecotopes. Since *T. sordida* and *T. pseudomaculata* live in more unstable ecotopes with fewer sources of blood they form small sparse colonies and invest more energy in reproduction than maintenance.

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VT-26 – MORPHOMETRIC AND MOLECULAR STUDIES OF INTRADOMICILIARY POPULATIONS OF *TRITOMA BRASILIENSIS* NEIVA, 1911

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The objective of this study was to determine the existence of genetic variability of populations of *T. brasiliensis* from intradomiciliary ecotopes, obtained in two States of the Brazilian Northeast (Ceará and Piauí). In Ceará, the insects were captured in two counties - Independência and Novo Oriente, and in Piauí, in the county of Simplício Mendes. The colonies of Independência and Simplício Mendes were founded starting from insects captured at several homes, and the one of Novo Oriente, of insects coming from just an intradomicílio. For the morphometric studies (Dujardin et al., 1998), were used heads of 15 males and 15 females of each population. For the molecular studies, using the RAPD ("Random Amplified Polymorphic DNA"), legs of 10 males and 10 females of each population were processed. The results of the morphometric studies indicate that the coming insects of Independência are larger, and that the males, analysed by they form, can be better differentiated than the females, suggesting the existence of a larger variability among them. The evolutionary tree built by the analysis of RAPD shows clear separation of the 3 populations, belonging the two of Ceará to a same group, and Simplício Mendes's population the other. Those results indicate the existence of great interpopulational heterogeneity in *T. brasiliensis*, that proceeds being investigated with insects from another states as well as from another ecotopes.

Financial Support: CPqRR/FIOCRUZ, FAPEMIG, ECLAT.

VT-27 – CHARACTERIZATION OF THE I UROTERGITE PROCESS IN *TRITOMA DELPONTEI*, ROMANÃ & ABALOS,1947 AND *TRITOMA PLATENSIS* NEIVA,1913 THROUGH ELECTRONIC MICROSCOPY AND OPTIC MICROSCOPY

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The I Urotergite Process has been characterized to Triatominae species from different places in Brazil and South America. Aiming to extend this study to other species of importance in the Chagas Disease, we are describing this structure to *Triatoma delponteii* which occurs in territories of Argentina, Uruguai and Paraguai and *Triatoma platensis* which is distributed in territories of Argentina, south of Bolivia and Paraguai. Adult males of *Triatoma delponteii* Romanã & Abalos 1947 from Santiago del Estero (CTA-129) were studied. On dorsal view *T.delponteii* presents the I Urotergite on a higher level than the II Urotergite as in *T. brasiliensis* and *T. rubrovaria* (Barata et al;1998).The triangular plate has a trapezium shape as in *T.infestans* (Barata et al;1994); its apex is rhomboid as in *T.sordida* (Barata et al. 1996 a,1996b) and it is lodged into a sunken surface which is in the circular area of the II Urotergite.. The I Urotergite presents a conspicuous transversal groove that is located at the bottom of this structure. Wrinkles are distinctly concentrated at the lateral and central parts of the I Urotergite; where plenty of long bristles can be seen. The circular area of the II Urotergite is smooth in almost its extension and presents in its central portion a sunken surface where the apex of the I urotergite is lodged. The circular area is limited by two prominent divergent, short, vertical grooves. The wrinkling of the remainder surface of the I Urotergite is scarcely visible and it is more evident on the sides. Bristles can't be seen at the Optic Microscope.*Triatoma platensis* Neiva, 1913 on dorsal view presents the I Urotergite on a higher level than the II Urotergite (Barata et al,1994,1996 a ,1996 b,1998). The triangular plate has a triangular shape whose sides are narrow and slightly concave, not reaching the adjacent angle of the II Urotergite as in the other species that have been studied. The I urotergite apex presents a downward pronounced slope and according to the geographical region, morphological changes were observed, as it was in *T.rubrovaria* (Barata et al,1998).While the sample from Cordoba has a pointed apex, the one from La Pampa has a rhomboid one. The II Urotergite usually presents a circular concave area limited by a pair of concentric, long, vertical grooves. In all extension of the rectangular plate wrinkles are distributed and they are more evident near the circular area. In the sample from La Pampa, the rectangular plate is extremely wrinkled. Long bristles are distributed in the circular area and on the lateral fringes and are plentifully spread in some specimens.

VT-28 – CHARACTERIZATION OF THE II UROTERGITE PROCESS IN *TRITOMA PROTRACTA* UHLER, 1894 AND *TRITOMA LECTICULARIA* STAL, 1859 THROUGH SCANNING ELECTRONIC MICROSCOPY AND OPTIC MICROSCOPY

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The I Urotergite Process was firstly described to some Triatominae species that are involved in the transmission of Chagas Disease at the South American Continent in areas of Bolivia, Argentina, Paraguai, Uruguai and Brazil. At this short communication ,

we are extending this characterization to two North American species that have been found naturally infected and can be associated to the enzootic cycle of this disease in Mexico middle south of the USA Adult males of *Triatoma protracta* Uhler,1894 from Monte Diablo CA (CTA-045) were studied. The triangular plate has an isosceles shape; it is on a higher level than the II Urotergite and display wrinkles and parallel, conspicuous grooves which take place in most of the structure. Its apex doesn't preserve an angular process as it was observed in the other species that have been studied. Conspicuous short bristles can be seen and they are distributed in the median portion of the I Urotergite. A different light coloured area distinguishes the apex border of the I Urotergite, including its apex. The rectangular plate presents a plane circular area well defined by a pair of divergent, vertical grooves. In the median portion of the circular area, not prominent parallel horizontal striations can be seen and in some specimens can also reach the adjacent portion of the II Urotergite. The II Urotergite is extremely wrinkled and striated, not showing bristles at the Optic Microscope. Adult males of *Triatoma lecticularia* from Oklahoma- USA (CTA-021) were studied. The triangular plate is on a higher level than the II Urotergite. At the middle of the plate there is a centre apical slope that is contiguous to the angular process and take the shape of a lozenge. This structure is distinctively seen in some specimens. Bristles are distributed at the central part of the plate and wrinkles are concentrated on the lateral fringes of the triangular plate. A wrinkly dark area surrounds the apex border of the I Urotergite including its apex. Parallel, vertical grooves are displayed at the base of the triangular plate. The II Urotergite presents a plane circular area well defined by a pair of divergent, vertical grooves. Wrinkles are distributed in all extension of the rectangular plate and the ones which are next to the border of the I Urotergite are more evident and darker than the others. Bristles could not be observed at the Optic Microscope.

VT-29 – MORPHOLOGICAL STUDY OF THE FORE WINGS OF *TRIATOMA GUAZU* LENT & WIGODZINSKY, 1979 (HEMIPTERA; REDUVIDAE; TRIATOMINAE) SEEN BY OPTICAL AND SCANNING ELECTRON MICROSCOPY (S.E.M.)

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Triatoma guazu, described with reference to only one specimen (female holotype), collected in Paraguay (Lent & Wigodzinsky, 1979, Bull. Am. Mus. Nat. Hist. 163:123-520) it received the specific name *guazu* (big in guarany), for being one of genus *Triatoma*'s largest representatives. In 1996, the male allotype, collected in Mato Grosso (Brazil), was described with particular attention to external genitalia (Lent et al., 1996, Mem. Inst. Oswaldo Cruz, 91 (3): 313-315). The objective of this work was to obtain additional morphological informations by the study of particular undescribed structures existing in the fore wings of *T. guazu*. Ten specimens (five males and five females) were provided by the Department of Entomology, National and International Laboratory of Reference in Taxonomy of Triatomines, Institute Oswaldo Cruz, were used. The fore wings were removed and submerged in 98% alcohol, mounted under coverslips using Canada of balsam for observation and drawings were made at X12 magnification using standard lucid camera Wild M5. For scanning electron microscope observation, the wings were dissected into pieces, individually fixed to a support, exhibiting dorsal and ventral surfaces, metalized with gold and photographed under various optical magnifications. Results indicated a corium with corrugated integument on dorsal surface of the wing, covered with spines, with setae and sensilla implanted among the spines. They were observed numerous setae surrounded by mechanoreceptors over the subcostal vein. In the middle of the membrane surface was evidenced a single campaniform sensillum. On the ventral surface, a ctenidium was observed, with a comblike structure made up of a row of flattened spines on the surface of the wing, as a part of a coaptor system, constituting an elementary component part of a morphological mechanism adapted for flight. This element is made up of thirty-three spines juxtaposed to each other, limited by a corrugated protuberance and a depression that allows the fitness of the hind wing. It is expected that the findings of these structures herein described will help further studies of comparative morphology and phylogeny of the group.

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VT-30 – DESCRIPTION OF THE EIGHTH AND NINTH UROSTERNITE OF MALE AND FEMALE FIFTH INSTAR NYMPHS OF *TRIATOMA GUAZU* LENT AND WYGODZINSKY, 1979 AND *TRIATOMA RUBROFASCIATA* (DE GEER, 1773) (HEMIPTERA, REDUVIDAE)

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Galliard (1935) defined the distinction of male and female fifth instar nymph of Triatominae. Rosa et alii suggested the possibility of Triatominae specific distinction in the fifth instar based on 8th and 9th urosternites caracteres. Afterwards three *Panstrongylus*, one *Rhodnius* and seventeen *Triatoma* species were studied. Now are described the 8th and 9th urosternite of fifth instar female and male nymph of *T. guazu* and *T. rubrofasciata*, through scanning electron microscope Topcon SM300. The male nymph of fifth instar of *T. guazu* shows a longitudinal swift depression across the ninth ventral segment, one pore in the posterior limit of this segment and one slit in the adjacent central portion with the tenth segment. The ninth abdominal ventral segment of female nymph of the *T. guazu* are united to the eighth segment in the central portion, shows (1+1) lateral slits and one "V" form depression in the adjacent tenth segment. The male nymph of fifth instar of the *T. rubrofasciata* shows one strong longitudinal depression across the ninth abdominal ventral segment and one depth slit in the adjoining portion with the tenth segment. The female fifth instar nymph of *T. rubrofasciata* has (1+1) projections in the eighth segment that covered part of the ninth abdominal ventral segment. The ninth segment of *T. rubrofasciata* has (1+1) lateral slits and one depression in the central portion. Another characteristic of this segment is the short dimension. The morphological structures of the ninth ventral abdominal segment of female fifth instar nymph of *T. guazu* and *T. rubrofasciata* are different of the following species: *T. arthurneivai*, *T. brasiliensis*, *T. circummaculata*, *T. costalimai*, *T. dimidiata*, *T. infestans*, *T. lecticularia*, *T. maculata*, *T. matogrossensis*, *T. platensis*, *T. pseudomaculata*, *T. protacta*, *T. rubrovaria*, *T. sordida*, *T. tibiamaculata*, *T. vitticeps* and *T. williami*.

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VT-31 – FLIGHT INITIATION IN *TRITOMA INFESTANS* (KLUG,1834) AND *T. MELANOSOMA* MARTINEZ, OLMEDO & CARCAVALLO, 1987 (HEMIPTERA, REDUVIIDAE)

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Domestic populations of *T. infestans* can be readily eliminated by residual spraying of infested houses using modern pyrethroid insecticides, but the treated premises then remain at risk to reinfestation by bugs coming from untreated foci. Dispersal of the bugs is frequently by passive carriage, for example amongst the clothes and utensils of people travelling between different villages, but the bugs can also disperse as adults flying between houses. For *T. infestans*, the mean effective range of adult flight seems to be around 200 metres (Schofield & Matthews 1985, *J. Trop. Med. Hyg.* 88: 211-222), although flights in excess of 1 Km have been recorded in the field (Schweigmann *et al.* 1988, *Med. Vet. Ent.* 2:401-404). Species occupying silvatic habitats may be expected to retain a greater capacity for active flight, as indicated by comparison of the flight capacity of silvatic *T. sordida* (Schofield *et al.* 1991, *Trans R. Soc. Trop. Med. Hyg.* 85: 676-678) with that of domestic *T. infestans* (Schofield *et al.* 1992, *Med. Vet. Ent.* 6: 51-56) under similar field conditions. We offer a further comparison of flight initiation, comparing *T. infestans* with the very closely-related *T. melanosoma*. Fifth instar nymphs of *T. infestans* and *T. melanosoma* were randomly chosen from the colonies maintained at Instituto Oswaldo Cruz, Rio de Janeiro. After moulting to adults, the bugs were individually placed in Borrel flasks, starved for 7 days, and then allowed to feed for 4 hours on restrained pigeons. Those that fully engorged were kept isolated for a further 14 days and then marked individually with paint on the thorax following the system of MacCord *et al.* (1983, *Mem. Inst. Oswaldo Cruz* 78:473-476). These individually marked bugs were then placed into a flight apparatus following the design of Lehane & Schofield (1982, *Bull. Ent. Res.* 72:497-510). Bugs were placed in the flight apparatus in the evening, and removed the following morning, so that a daily record was maintained of flight until all bugs had died. In total, observations were made on 46 specimens of *T. melanosoma* and 50 of *T. infestans*, although the number of bugs present in each flight apparatus on each occasion varied from 1 to 18 because of their different emergence times. Most of the bugs initiated flight within the first 5 days of observation. During this time, 70% of male and 85% of female *T. infestans* initiated at least one flight, compared to 77% of male and 65% of female *T. melanosoma*. The proportion of flights then steadily declined with time in both species, although females continued to initiate flight more frequently than males. During the whole period of observation up to death of the final adult (183 days for *T. infestans*, 232 days for *T. melanosoma*) females of *T. melanosoma* flew on 57% of occasions and females of *T. infestans* flew on 46% of occasions, whereas males of the two species flew on 47% and 39% of occasions, respectively. Similarly, 96% of female *T. melanosoma* and 87% of female *T. infestans* initiated at least one flight during the observation period, compared to 90% of male *T. melanosoma* and 74% of male *T. infestans*. However, none of these differences is significant (Mann-Whitney test) either between species or between sexes. Our results confirm those of earlier observations on *T. infestans* (Lehane & Schofield, 1982 *op.cit.*) showing that flight initiation generally declines from a peak at about 14 days post-feeding, and is generally more marked in females than in males – though rarely significantly so.

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VT-32 – MORPHOMETRIC STUDIES OF LEGS OF 1ST AND 2ND INSTAR NYMPHS OF *MEPRAIA SPINOLAI* AND *TRITOMA INFESTANS* (HEMIPTERA, REDUVIIDAE)

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Chagas's disease occurs in 17 countries of Latin America and the most important mechanism of *Trypanosoma cruzi* man transmission is Triatominae faeces, being *T. infestans* the main vector (Silveira; 1983; OMS, 1991). *M. spinolai*, wild species belonging to Triatominae subfamily is unique in *Mepraia* genus, and unique in Triatominae subfamily to have males macropterous, brachypterous, or micropterous and females micropterous. Another characteristic of this species is to have long and slender legs, mainly those of 3rd pair (Lent and Wygodzinsky, 1979). In this study, the segments of the legs of the three pairs of 1st and 2nd instars nymphs of *Mepraia spinolai* and *Triatoma infestans* were measured by Nikon profile projector model 6C. Statistical analysis of the segments of the legs measured was done by INSTAT program, where we obtained average, standard deviation and standard error. Significant difference was not observed between right and left segments of the legs of 1st and 2nd instars nymphs of *Mepraia spinolai* and *Triatoma infestans*. Second instars nymphs of *Triatoma infestans* showed segments of legs significantly bigger than the 1st instars nymphs. The segments of the legs of the 1st instars nymphs of *Mepraia spinolai* presented the following measurement differences: tīb) fêm) tar) tro) cox, while the 2nd instars nymphs showed tīb) fêm) tar) cox) tro. The 2nd and 3rd pairs of legs of the 1st and 2nd instar nymphs of *Triatoma infestans* showed tīb) fêm) tar) tro) cox, while in 1st pair of legs the relation was fêm) tīb) tar) tro) cox. *Mepraia spinolai*'s femur and tibia are bigger than *Triatoma infestans*' ones in both instars.

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VT-33 – WEIGHT REDUCTION OF EACH INSTAR OF *TRITOMA JURBERGI* CARCAVALLO, GALVÃO & LENT, 1998, (HEMIPTERA, REDUVIIDAE) PERMITTED ONLY ONE BLOODMEAL

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Triatoma jurbergi, a closely-related species of *Triatoma guazu* Lent & Wygodzinsky, 1979, was described recently by Carcavallo *et al.* (1998, *Mem. Inst. Oswaldo Cruz*, 93(4):459-464 based on specimens collected in Rondonópolis, Mato Grosso, Brazil. It is a sylvatic species, that has been found occasionally in the surroundings of rural houses, however, its role in the epidemiology of the Chagas' disease not yet established. The objective of the present work is to assess losses of weight for each evaluated instar after a single bloodmeal. Reared insects were obtained from the Triatominae International Reference Laboratory at the Department of Entomology of Instituto Oswaldo Cruz Rio de Janeiro, Brasil. Fifty eggs and 50 specimens of each instar were randomly removed from the rearing colony, individually enclosed in Borrel flasks and maintained under environmentally controlled conditions. Insects were fed on pigeons (*Columba livia*) as protein food source. Soon after eclosion and/or moult, insects were weighed and allowed to have only one bloodmeal. After each bloodmeal, insects were individually weighed again and weighing every seven days thereafter until next ecdysis for death. Results on body weight of insects submitted to different bloodmeals and weight losses during each phase of development (table included). Indicated a longer life span period the second instar, in spite of the fact that these nymphs ingested, proportionally, lesser amount of blood.

	Inicial Weight (mg)	Ingested Blood (mg)	Weight after Feeding (mg)	Losses of Weight (mg)	Survival Period
1 ^o instar	X=1,6+/-0,6	X= 6+/-3	X=6+/-3	X=4+/-0,1	X=35+/-18
2 ^o instar	X=5+/-1,7	X=21+/-9	X=29+/-9	X=14+/-5	X=49+/-24

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VT-34 – *TRITOMA INFESTANS* SALIVA APYRASES ARE 480 KDA OLIGOMERS

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Triatoma infestans feeding ability, here understood as its capacity to overcome the host homeostatic response, can be related to various compounds secreted in its saliva. In a previous study, we identified and characterized apyrase activity in the saliva of *T. infestans*, the main vector of Chagas' disease in Brazil. The apyrase, a diphosphohydrolase that removes Pi from ATP and ADP, is a potent platelet anti-aggregator, which has been associated with fluidity of the blood essential for the insect feeding and thriving.

This activity was purified by gel filtration chromatography as a 480 kDa protein. The two dimensional electrophoresis analyses showed the presence of isoenzymes composed of peptide chains of 79, 82 and 88 kDa. The Peptide Mass Fingerprinting technique showed the 79 and 88 kDa polypeptides to be homologous to the *Aedes aegypti* apyrase. Cross-linking experiment with glutaraldehyde demonstrated those chains are assembled in a high molecular mass complex. The purified apyrase molecular complex was submitted to native PAGE and formed a single 460 kDa band under silver staining, which co-migrates with the in-gel apyrase activity. These findings show that this polypeptide oligomer encloses all apyrase activity in the saliva of *T. infestans*.

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VT-35 – STUDY OF ENZYMES IN TESTES AND OVARIES FROM ADULT *DIPETALOGASTER MAXIMUS* AND *TRITOMA INFESTANS*. CORRELATION WITH ULTRASTRUCTURE

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D. maximus and *T. infestans* are vectors of *Trypanosoma cruzi*, the etiological agent of American trypanosomiasis. Knowledge of functional aspects of organs involved in reproduction of these species may be useful for the design of control methods. Activity of enzymes related to glycolysis: hexokinase (HK), fructose-6-phosphate kinase (F6PK); pentose pathway: glucose-6-phosphate dehydrogenase (G6PDH); amino acid catabolism: glutamate dehydrogenase (GlutDH) and involved in shuttle systems for the transfer of reducing equivalents to the mitochondria: aspartate aminotransferase (AAT), malate dehydrogenase (MDH) and glycerol-3-phosphate dehydrogenase (GPDH) were determined on extracts of adult testes and ovaries from *D. maximus* and *T. infestans*. The fine structure organization of the same organs were studied by electron microscopy. Testes: Activities of F6PK in *D. maximus* and *T. infestans*, either expressed in U/mg protein or U/g wet tissue, were higher than that of G6PDH. HK activity was lower than F6PK in *D. maximus*; in contrast, HK was higher than F6PK in *T. infestans*. The activity of F6PK was twice as high as GlutDH in both species. AAT and MDH showed higher activity than GPDH. Ovaries: F6PK showed similar activity to G6PDH. At variance with *T. infestans*, HK activity in *D. maximus* was lower than that for F6PK. GlutDH was higher than F6PK in both species. AAT and MDH activities were greater than that of GPDH. Levels of enzymes would be an index of the activity of the pathways in which those enzymes are integrated. In testes from both species most of the glucose would be utilized through the glycolytic pathway. Number of mitochondria and development of the rough endoplasmic reticulum in cell of the spermatogenic line indicate very active oxidative metabolism and protein synthesis. In ovaries, the existence of an active pentose pathway supply the NADPH required for fat and ecdysteroids synthesis. Catabolism of amino acid ending in alpha-ketoglutarate appears to be relatively more important in ovary than in testis. Fat and glycogen are stored in follicular cells of *D. maximus*; oocytes of both species contain numerous fat droplets. Abundant mitochondria are present in follicular cells and oocytes. Rough endoplasmic reticulum and free ribosomes are also conspicuous in these cells. The malate/aspartate H-transfer system seems to be relatively more important than the glycerophosphate shuttle in ovaries as well as in testes, at variance with our finding in muscles from the same insects (*Comp. Biochem. Physiol* 116A:173-179, 1997)

VT-36 – STUDY ON THE FEEDING BEHAVIOR OF *TRITOMA SORDIDA* (STAL 1859) (HEMIPTERA – REDUVIDAE) CAPTURED IN MATO GROSSO STATE BRAZIL USING THE PRECIPITIN TECHNIQUE AND DEGREE OF INFECTIVITY

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The authors assessed using precipitin technique, food source of two hundred thirty four specimens of *Triatoma sordida* (Stal, 1859) captured in domiciliar and peri-domiciliar environment of Rondonópolis, Mato Grosso State, Brazil. The series of anti-serum used and respective titles were as follows: Anti-human 1:15.000, anti-bird 1:10.000, anti-dog 1:15.000, anti-cat 1:12.000, anti-horse 1:16.000, anti-goat 1:14.000, anti-cow 1:15.000, anti-pig 1:10.000, anti-rodent 1:17.000, anti-opossum 1:15.000, anti-armadillo 1:15.000. Natural infection was assessed simultaneously using standard microscope examination.

Results indicated that one hundred twenty eight specimens reacted with following food source, respectively: goat (13.7%), opossum (marsupial) (13.7%), rodent (10.7%), bird (6.4%), dog (3%), pig (3%), human (2.6%), lizard (0.85%), frog (0.85%). One hundred and six specimens reacted with more than one food source as follows: rodent-opossum (7.3%), goat-bird (5.1%), goat-pig (2.6%), bird-frog (2.6%), bird-opossum (2.6%), bird-rodent (1.7%), frog-opossum (1.7%), bird-lizard (1.7%), opossum-pig (1.7%), goat-pig (1.3%), opossum-goat (1.3%), dog-opossum (1.3%), dog-frog (0.85%), lizard-bird (0.85%), dog-goat (0.85%), pig-lizard (0.85%), rodent-lizard (0.85%), pig-bird (0.85%), human-opossum (0.85%), pig-rodent (0.43%), frog-pig (0.43%), lizard-goat (0.43%), dog-bird (0.43%), rodent-lizard (0.43%), goat-rodent-opossum (1.7%), opossum-bird-rodent (0.85%), bird-opossum-goat (0.85%), rodent-bird-goat (0.85%), opossum-goat-dog (0.43%), goat-frog-bird (0.43%) rodent-bird-pig (0.43%), opossum-goat-rodent (0.43%), pig-bird-opossum (0.43%), rodent-opossum-goat-bird (0.85%) and bird-rodent-goat-lizard (0.43%).

None of the simples reacted with anti-cat, anti-horse, anti-cow neither anti-armadillo. Bigger incidence was observed in opossum and goat followed by rodent and bird. On the other hand natural infection for *Trypanosoma cruzi* like was detected in one hundred five (44.9%) specimens.

Trabalho realizado com auxílio do convênio N.123/97 Fiocruz/FNS;CNPq; ECLAT – European Community Latin America Triatominae Research Network IC 18- CT- 960054

VT-37 – FEEDING STUDY OF SOME SPECIES OF TRIATOMINAE CAPTURED IN RIO GRANDE DO SUL STATE, BRAZIL, USING THE PRECIPITIN TECHNIQUE AND DEGREE OF THE INFECTIVITY

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3- Gerência Nacional do programa de controle da Doença de Chagas – FNS – Brasília – D.F.

The objective of the work was to make a comparative feeding study of some species of Triatominae using the technique of precipitin, and natural infection was assessed using standard microscope examination.

The following species and number of individuals, respectively, were assessed: *Triatoma infestans*, 81 specimens; *Panstrongylus megistus*, 78 specimens; *P.tupynambai*, 7 specimens and *Triatoma rubrovaria*, 271 specimens, all captured in the state of Rio Grande do Sul, Brazil.

A Batery of anti-serum and respective titles used were as follows: anti-human 1:15.000, anti-bird 1:10.000, anti-dog 1:15.000, anti-cat 1:12.000, anti-horse 1:16.000, anti-goat 1:14.000, anti-cow 1:15.000, anti-pig 1:10.000, anti-rodent 1:17.000, anti-opossum 1:15.000, anti-armadillo 1:15.000 e anti-sheep 1:8.000.

Results indicated that 27 specimes of *T.infestans* fed on rodents 9.9%, birds 9.9%, humans 2.5% and horses 2.5% and 52 reacted with more them one food source birds-rodents 19.7%, birds-opussums 19.7%, rodents-opussums 12.3% and birds-rodents-opossums 3.7%. 61 specimes of the *P.megistus*, birds 19.2%, humans 15.4%, dogs 14.1% and horses 11.5%,. 17 specimes reacted with more them one food source, rodents-birds 5.12%, humans-birds 2.6%, birds-dogs 2.6%, dogs-rodents 2.6%, birds-dogs-horses 1.3% and humans-birds-rodents-opossums 1.3%. 3 specimes of the *P. tupynambai* fed on: dogs 28.5%, rodents 14.3%, 4 specimes reacted with more them one food source: humans-rodents 14.3%, birds-rodents 14.3%, humans-dogs 14.3%, birds-dogs-rodents 14.3%. 189 specimes of *T.rubrovaria* fed on rodents 19.5%, birds 16.2%, dogs 12.1% and humans 7.0%. 75 specimes reacted with more them one food source as follows: dogs-rodents 3.7%, humans-birds 3.7%, humans-rodents 1.8%, humans-birds-dogs 1.1% and rodents-humans-birds 0.36%.

Results of those naturally infected by *Trypanosoma cruzi* like indicated 33 (40%) individuals of *Triatoma infestans* positive, 32 (39.7%) of *Triatoma megistus* positive, 1 (14.3%) specimen of *P.tupynambai* positive and 52 (19.2%) specimens of *T.rubrovaria* positive.

Trabalho realizado com auxílio do convênio N.123/97 Fiocruz/FNS;CNPq; ECLAT – European Communit Latin America Triatominae Research Network IC 18- CT- 960054

VT-38 – MORPHOMETRIC STUDIES OF ANTENNAL SEGMENTS OF NYMPHS AND ADULTS OF PANSTRONGYLUS MEGISTUS AND RHODNIUS NEGLECTUS (HEMIPTERA, REDUVIIDAE)

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The Chagas disease affect a universe of 16 million of people in Latin America (WHO, 1991). Five million of people in Brazil are infected by *Trypanosoma cruzi* (Dias, 1987). These index show us the importance and necessity of the Triatominae studies. The relative lenght of the four antennal segments are used as taxonomic character (Lent & Wygodzinsky, 1979). Fifteen samples of *P. megistus* and *R. neglectus* were utilized for the mensuration of four right and left antennal segments of the first to the fourth instar nymphs and, male and female fifth instar nymphs and adults, through Nikon profile projector model 6C. The measurements were statistically evaluated by the INSTAT program through ANOVA test, t-student paired test and t-student non paired test. Significant and diferent remarks were observed between the four antennal segments of five instar nymphs and adults. The following results were obtained for the first and second instars nymphs of *P. megistus*: 4th>3rd>2nd>1st, for the third instar nymphs: 3rd>4th>2nd>1st, for fourth and fifth instars nymphs and adults : 2nd>3rd>4th>1st. The differences noted between antennal segments of *R. neglectus* were: in first and second instars nymphs: 3rd>2nd>4th>1st, in the third and fourth instar nymphs: 3rd>2nd>4th>1st and fifth instar nymphs and adults: 2nd>3rd>4th>1st. Significant diference was not observed between right and left segments, between male and female in both species. Based on the results of *P.megistus* we could note and compare the similarity with *T. rubrovaria* (Tres et alii, 1996, 1997). The research measurement data of antennal segments of *R. neglectus* are similar to *T. rubrovaria* and *P. megistus* in the fifth instar nymphs and adults, on the others instars standard measure are distincts.

Supported by CNPq.

VT-39 – CHARACTERIZATION OF THE II UROTERGITE PROCESS IN *TRITOMA PROTRACTA* UHLER, 1894 AND *TRITOMA LECTICULARIA* STAL, 1859 THROUGH SCANNING ELECTRONIC MICROSCOPY AND OPTIC MICROSCOPY

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The I Urotergite Process was firstly described to some Triatominae species that are involved in the transmission of Chagas Disease at the South American Continent in areas of Bolivia, Argentina, Paraguai, Uruguai and Brazil. At this short communication,

we are extending this characterization to two North American species that have been found naturally infected and can be associated to the enzootic cycle of this disease in Mexico middle south of the USA. Adult males of *Triatoma protracta* Uhler, 1894 from Monte Diablo CA (CTA-045) were studied. The triangular plate has an isosceles shape; it is on a higher level than the II Urotergite and display wrinkles and parallel, conspicuous grooves which take place in most of the structure. Its apex doesn't preserve an angular process as it was observed in the other species that have been studied. Conspicuous short bristles can be seen and they are distributed in the median portion of the I Urotergite. A different light coloured area distinguishes the apex border of the I Urotergite, including its apex. The rectangular plate presents a plane circular area well defined by a pair of divergent, vertical grooves. In the median portion of the circular area, not prominent parallel horizontal striations can be seen and in some specimens can also reach the adjacent portion of the II Urotergite. The II Urotergite is extremely wrinkled and striated, not showing bristles at the Optic Microscope. Adult males of *Triatoma lecticularia* from Oklahoma- USA (CTA-021) were studied. The triangular plate is on a higher level than the II Urotergite. At the middle of the plate there is a centre apical slope that is contiguous to the angular process and take the shape of a lozenge. This structure is distinctively seen in some specimens. Bristles are distributed at the central part of the plate and wrinkles are concentrated on the lateral fringes of the triangular plate. A wrinkly dark area surrounds the apex border of the I Urotergite including its apex. Parallel, vertical grooves are displayed at the base of the triangular plate. The II Urotergite presents a plane circular area well defined by a pair of divergent, vertical grooves. Wrinkles are distributed in all extension of the rectangular plate and the ones which are next to the border of the I Urotergite are more evident and darker than the others. Bristles could not be observed at the Optic Microscope.

VT-40 – MORPHOMETRICS OF THE GENUS *PANSTRONGYLUS* BERG, 1879 (HEMIPTERA, REDUVIIDAE, TRIATOMINAE)

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The genus *Panstrongylus* Berg, 1879, is composed by 13 species, all have a neotropical distribution; they are prevalent from the South of Mexico to the Patagonian region in Southern Argentina (Carcavallo et al., 1994, *Entomol. Vect.*, 1: 113-120). All species of this genus have been described in detail through the study of external and internal morphology, showing significant differences between male genitalia (Lent & Jurberg, 1968, *Rev. Brasil. Biol.*, 28: 499-520; Lent & Jurberg, 1975, *Rev. Brasil. Biol.*, 35: 379-438; Lent & Wygodzinsky, 1979, *Bull. Am. Mus. Nat. Hist.* 163: 127-130). Some of them have importance as vectors' of *Trypanosoma cruzi*, the causative agent of Chagas' disease, such as *P. megistus* Burmeister, 1835 and *P. geniculatus* (Latreille, 1811) (Curto de Casas et al., 1996, *Entomol. Vect.* 3 (2): 43-58). Morphometry is suggested here as a new "tool" capable not only of solving taxonomic problems about Triatominae, but also to try to create a phylogenetic approach. Recent works by Dujardin et al. (1999, *Mem. Inst. Oswaldo Cruz* 94: 565-569) suggest that changes in the sexual size dimorphism of Triatominae can be used as a new character to study the species of Triatominae in the transition from natural to artificial habitats, like domestic ecotopes. The present work uses all species of *Panstrongylus*, deposited in the "Herman Lent" collection of the Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos, Departamento de Entomologia, Instituto Oswaldo Cruz, FIOCRUZ, Brasil. The measurements were made using a stereoscopic microscope ("Wild M5-51844"), with micrometer eyepiece; 13 measurements were taken from the head, of each adult specimen, as described by Lent & Wygodzinsky (1979, *Bull. Am. Mus. Nat. Hist.* 163: 127-130) important in the taxonomy of the Triatominae. To this date 56 specimens have been measured: 9 of *P. chinai* (Del Ponte, 1929); 4 of *P. humeralis* (Usinger, 1939); 4 of *P. lignarius* (Walker, 1873); 29 *P. megistus* and 10 of *P. rufotuberculatus* (Champion, 1899), from different places. The preliminary results corroborate the sexual dimorphism already known, but in *P. megistus* these differences were more obvious than in the other species because the females were significantly larger than males, from different localities, in all the measured variables, suggesting a possible populational variability.

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VT-41 – COLONIZATION OF *LUTZOMYIA LONGIPALPIS*: MORPHOLOGICAL STUDY AND SUSCEPTIBILITY TO INFECTION BY *LEISHMANIA SPP.*

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A colony of the phlebotomine *Lutzomyia longipalpis* from the Lapinha cave (Minas Gerais state, Brazil) was established in a short time in our Laboratory. The intention was to obtain enough flies to develop several experiments as: (a) to study the aspects of each developmental stage of the sandfly; (b) to study the morphology of the midgut and (c) to investigate the susceptibility of the sandfly to the *Leishmania* infections. The colony started with 850 adult females from the wild life, caught during nighttime with CDC light traps. After the females came from the field, they were blood fed in anesthetized hamster for the next four days. The engorged females were separated and individualized for oviposition and identification. The eggs were counted, separated and placed in pots of plaster for creation. The larvae were daily fed on a special diet. The insects were all time maintained at 26° C in a B.O.D. incubator. The adult releases were completed in a period of about 30-35 days. After this, the colony was established and maintained until now, the 11th generation. In following experiments we used the scanning electron microscopy to observe the eggs and larvae morphology. The external surfaces of the eggs are covered with an exochorion characterized by arrangements of a series of parallel, discontinuous, longitudinal ridges that converge at egg ends. There are not lateral connections between the ridges. The larvae stages were distinguished by the presence of one or two pairs of caudal filaments. The abdominal segments were recognized by the presence of stump projecting structures on the dorsal surface called prolegs. The lateroventral surface is covered with different brush-like hairs. In the pupae stage is easy to observe the abdominal segments with lot of spiculates, the wings and the legs. We also developed a histological study of the adult midgut, the region where the *Leishmania* parasites initiate the infection. Conventional staining of Histoiresin sections showed details of the organ, demonstrating differences between the thoracic and abdominal regions of the midgut. Another experiments were done in order to verify the infection of the *Lu. longipalpis* by *Leishmania*. We verified that insects from the colony are much more susceptible to infection than those from the wild life. In order to better understand this aspect we developed biochemistry studies to compare the proteins present in the midgut and in the saliva of these insects. We concluded that they are also different in the protein contents of these organs. Further studies are necessary to understand these aspects and possible relationship with the parasite-vector interaction.

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VT-42 – MOLECULAR POLYMORPHISM WITHIN A PUTATIVE SONG GENE OF *LUTZOMYIA LONGIPALPIS*

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During courtship, males of *Drosophila* produce a “lovesong” by vibrating their wings, and this species-specific acoustic signal has been implicated in the reproductive isolation of closely related species. Any gene that influences the characteristics of this courtship song is therefore a candidate for influencing the speciation process. *cacophony* (*cac*) is a calcium-channel gene that determines particular features of the courtship song in *Drosophila melanogaster*. *cac* is particularly interesting from an evolutionary point of view because one of its mutant alleles produces a song that resembles those of other *Drosophila* species.

The sandfly *Lutzomyia longipalpis* (Psychodidae; Phlebotominae), the vector of visceral leishmaniasis in Latin America, is considered a complex of sibling species. Courtship songs have been recorded from *L. longipalpis* and, as in *Drosophilids*, it is possible that they play a role in the reproductive isolation of sandflies. As part of a molecular and behavioural analysis of courtship songs in neotropical sandflies, we recently isolated and sequenced from *L. longipalpis*, a DNA fragment homologous to *cacophony*. We are now analysing the molecular polymorphism within this putative sandfly song gene using PCR and DNA sequencing. A number of alleles were sequenced from two populations of *L. longipalpis* (Natal, RN and Lapinha, MG) and preliminary analysis suggests that *cacophony* will be a good molecular marker for speciation studies within this species complex.

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VT-43 – TRANSIENT EXPRESSION OF DIFFERENT GENES IN CELL LINE AND MIDGUT OF *LUTZOMYIA LONGIPALPIS*

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Genetic manipulation of insect vectors, in order to alter their ability to transmit parasites, may provide a new approach in the control of vector-borne diseases. Sandfly midgut plays an important role in the transmission of *Leishmania* parasites. The ability to express genes in sandfly would be a powerful approach to characterize midgut genes, and to reveal important vector determinants of pathogen transmission. In the present study, we report a method for the transfection of midguts from *Lutzomyia longipalpis*, the *Leishmania chagasi* vector. Midguts are important for parasite metacyclogenesis, and as such, the prime target for the expression of deleterious genes to the parasite. A lipofectin-based assay was used to transfect plasmids carrying the luciferase or β -galactosidase reporter genes under control of the *Drosophila* heat-shock protein 70 promoter. Indirect immunofluorescence was used to characterize the luciferase expression at different plasmid concentrations and times post-transfection. The guts viability in 24 h culture was tested by *Leishmania* promastigotes binding assays *in vitro* and by ³⁵S methionine incorporation. The number of parasites that remained bound to transfected guts and the protein synthesis were comparable to the controls.

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VT-44 – EFFECT OF A SECOND BLOOD-MEAL OF *LUTZOMYIA MIGONEI* (DIPTERA:PSYCHODIDAE) ON DEVELOPMENT OF *LEISHMANIA BRAZILIENSIS*

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A few experimental studies have shown the effects of vector physiology on the life cycle of *Leishmania* parasites (Killick-Kendrick, R., 1979, Biology of Kinetoplastida, vol. 2, Academic Press, 395-460). In the present laboratory study we demonstrated that a second sand fly bloodmeal induces the multiplication and differentiation of *Leishmania* in early development. *Leishmania* promastigotes undergo sequential steps of proliferation and differentiation within their sand fly vectors to produce a final stage infective to the vertebrate host. Infection of laboratory-reared *Lutzomyia migonei* was carried out using artificial feeders containing a suspension of 2×10^7 *Le. braziliensis* amastigotes per ml of mouse blood. Approximately 300 female sand flies divided among 5 experimental groups were allowed to feed through chick-skin membranes stretched over the mouth of the feeders, which were placed over plastic rearing containers. Each sand fly was estimated to ingest approximately 2000 parasites. A second bloodmeal was offered on anesthetized, uninfected hamsters at 4 days after the infection. The re-fed females were counted and isolated in a separate cage from the unfed insects, the latter being used as a control. Both groups were provided with fresh sucrose solution and examined for *Leishmania* development. Females were dissected daily from 1-10 days after infection and their guts examined for presence of promastigotes under phase contrast microscopy, the numbers of parasites being counted in a hemacytometer. In addition Giemsa-stained smears were used to observe each of the different promastigote forms of *Leishmania*. The sand flies dissected 4 days after the infection (n=975) a total of 407 females had engorged or taken a partial bloodmeal within the 5 experimental groups, an average re-feeding rate of $55\% \pm 12.4$ (range=37-75). The results showed that the second blood meal caused no deleterious effects on *Leishmania*-infected sand flies. An increase in parasite density, with a slight change in the early development was detected in the females that took the second blood-meal. Similar proportions of the different promastigote forms of the parasites were noted in both groups. An increase in the proportion of the metacyclic forms in the proboscis would increase the efficiency of transmission after the intake of the second blood-meal.

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VT-45 – COMPARATIVE MOLECULAR STUDIES OF GUTS FROM FED AND UNFED SAND FLIES

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One of the possible approaches for the control of insect-borne diseases is through the control of the vector. Besides the traditional methods involving insecticides, recent developments, mostly related to mosquitoes, aim at the generation of insects less fit to transmit diseases. A first step necessary in the development of these studies is the basic knowledge of molecules involved in feeding mechanisms, and eventually in the parasite/host interactions. We have initiated studies on the characterization of such molecules in sand flies.

Gut of sand flies unfed or fed on rabbit blood were compared molecularly through differential display reverse transcription (DDRT) PCR employing various combinations of anchored oligo dT plus arbitrary primers (APs). Specific bands were excised from the acrylamide gel, the DNA eluted, reamplified by PCR and cloned, either using the pCR 2.1 TOPO vector (TOPO TA cloning kit, Stratagene) or the pBluescript plasmid digested with Hind III. We have also applied RAPD-PCR (using OPG primers kindly donated by Dr. Edelberto Dias, CPqRR, FioCruz) to amplify RNAs from unfed and fed guts as well as from salivary glands. With this latter approach we have also obtained a different pattern of bands which were separated on agarose gel, purified and cloned.

Upon sequencing of several clones we detected one that displayed a motif found to be homologous to an *Anopheles gambiae* gut-specific chitinase. When this clone was used as a probe in a Northern blot, containing whole insects RNA from blood-fed and sugar-fed *Lutzomyia longipalpis*, a signal was detected only against those that were fed on blood. Furthermore, this signal appeared only at approximately 30 hours after feeding with no signal detected at 16 hours after feeding, demonstrating the specificity of expression of this chitinase. Due to the likely fundamental physiological role a gut-specific chitinase may play in this vector, this and other clones obtained are being further characterized.

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VT-46 – RESEARCH ON PHLEBOTOMINAE (DIPTERA: PSYCHODIDAE) IN GRUMARI, AN AREA OF ENVIRONMENTAL PROTECTION OF THE COUNTY OF RIO DE JANEIRO, RJ, BRAZIL

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Grumari is an area of environmental protection, located in the Jacarepagua lowlands, a place in the west zone of the county of Rio de Janeiro, between Recreio e Barra de Guaratiba, with the Atlantic Ocean in the south. This area belongs to XXIV Administrative Regional Office of Barra da Tijuca. In September 1998, there occurred an autochthon case of visceral leishmaniasis. With an aim to investigate and keep updated the Phlebotominae fauna, as well as promoting a more effective entomological observation, mainly concerning the evaluation of the watering of insecticidal products, 9 (nine) captures were done in 4 distinct areas. Three of took us 4 hours and the others, for which we used a CDC trap, took us 12 hours, from November 1998 till May 1999.

A total of 5,810 sandflies were captured and identified, such as: *L. intermedia*, *L. migonei*, *L. firmatoi*, *L. schreiberi*, *Lutzomyia (Pintomyia) sp.* and *Brumptomyia cunhai*. The most abundant species was the *L. migonei* with 54,81% of samples, which, due to its anthropophily, makes it possible to see it as vectors of this disease in the meridional region of the South-American Continent (Forattini, 1973). The *L. intermedia* was 44,5% out of the sandflies collected. This species is suspected to be the vector of LTA in the Southeast region (Rangel et al., 1984; 1986; Rangel, 1995). It should be pointed out that no samples of *L. longipalpis* were captured during this period, though the visceral leishmaniasis case mentioned above and 10 positive dogs in the region were recorded.

VT-47 – ACTION OF HUMAN SERA AND VENEZA ZONATA (HEMIPTERA, COREIDAE) HAEMOLYMPH ON CULTURE FORMS OF TRYPANOSOMATIDS

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The interaction of Trypanosomatids with phytophagous hemipterans can define the localization of the flagellates in the insects (digestive tract and/or salivary glands) and classify the trypanosomatids as monoxenous (parasites of insects) or heteroxenous (involving parasites of insects and plants). The action of human sera and *Veneza zonata* haemolymph was analyzed on 3 groups of Trypanosomatids. The first group was composed by the trypanosomatids *Phytomonas serpens*, *P. mcgheeii* and the strain 714 TD, isolated from digestive tract of *V. zonata*, all of them establishing infection in the salivary glands of the insect *V. zonata*, and transmitting infection to plants. The second group (*Leishmania (L.) amazonensis*) represents trypanosomatids that could not establish an infection in *V. zonata*. The third group represents the strain 563 TD, a trypanosomatid isolated from the digestive tract of the phytophagous hemipteran *Piezodorus guildini*, pathogenic to the *V. zonata*.

The experiments with human sera used 30mL of undiluted normal sera mixed with 30mL of culture forms of trypanosomatids (10⁶ cells/mL) in Eppendorf tubes, incubated at 25°C and aliquots examined after periods of time, directly and stained with May-Grünwald-Giemsa. Almost immediately after the contact with human serum, the flagellates suffer strong agglutination. Groups 1 and 3 showed flagella-flagella, body-body and body-flagella interactions, whereas group 2 (*Leishmania (L.) amazonensis*) showed a faster flagellar agglutination.

With *Veneza zonata* haemolymph, collected with automatic pipette (30mL) from a leg of *V. zonata*, was processed as with serum samples. The results of group 1 showed a recognition of protozoa by haemocytes in 5 minutes and at 35 minutes it was seen several nodules, without agglutination and in 1 hour, several culture forms presents round forms. *L. (L.) amazonensis* seemed to be more sensitive than isolates from group 1. The flagellates adhered to haemocytes at time 0, with much more intense agglutination and at time 10 min., it was observed large rosettes of flagellates and at time 30 minutes, including haemocytes. The group 3 showed no significant differences from group 1. The morphologic alteration is studied by electronic microscopy and fenoloxidase biochemistry.

Financial Support: CAPES, CNPq, CPG/UEL

VT-48 – CULICOIDES MIDGES (DIPTERA: CERATOPOGONIDAE) FROM LOCALITY OF CAMPOS DOS GOYTACAZES, RIO DE JANEIRO, BRAZIL

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The ceratopogonid midges of the genus *Culicoides* are common bloodsuckers, which have been involved in the transmission of human and animal diseases. They are known to serve as vector of protozoan and nematode parasites to birds and mammals, and virus to humans.

At present, 246 valid species were assigned for the Neotropical Region. Only 66 have been cited south of the Amazonian Region. Very little work with systematical capture has been done in the Neotropical Region if compared with mosquitoes and other nematoceros diptera and more studies are necessary to best know the distribution of the Brazilian species.

We have had the opportunity to study material from light trap collection made by Fundação Nacional de Engenharia e Meio Ambiente (FEEMA) in 69 localities of the state of Rio de Janeiro, Brazil, during February 1989 to March 1990. First, we have studied the locality of Campos dos Goytacazes. After examination of 27 of the 50 total samples, we have obtained 75 specimens corresponding to the period of February to September 1989. The proportion of the species represented in these collections is as follows: *C. limai* group (47%), *C. insignis* (17,5%), *C. pusillus* group (17%), *C. guyanensis* (8%), *C. guttatus* group (4%), *C. foxi* (1,5%) and *C. neoparaensis* (1,5%).

At present, *Culicoides* of the *limai* group is more frequently found in this Region, considering this method of capture. *Culicoides insignis* and *pusillus* were also found in significant number. These last two species have been incriminated in the transmission of the bluetongue virus on cattle in the Central and South America.

* Programa de Vocaç o Cient fica da Funda o Oswaldo Cruz (PROVOC).

VT-49 – A HEME-BINDING ASPARTIC PROTEINASE FROM THE EGGS OF THE HARD TICK *BOOPHILUS MICROPLUS*

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The hard tick *Boophilus microplus* represents a major problem to cattle raising in tropical and subtropical areas of the globe, being the vector of many important cattle parasites such as *Babesia bovis* and *Babesia bigemina*. We have purified a new aspartic proteinase from the eggs of this tick. This proteinase is able to bind heme with a 1:1 stoichiometry. The proteinase, herein named THAP (Tick Heme-binding Aspartic Proteinase), was classified as an aspartic proteinase based on its NH₂-terminal sequence, acidic optimum pH and inhibition by pepstatin.

THAP showed hydrolytic activity against hemoglobin. However, very low activity was observed against globin devoid of the heme prosthetic group. Hydrolysis of globin polypeptide chain by THAP increased as increasing amounts of heme were added to globin, with maximum activation at a heme:globin relation of 1:1. Further additions of heme to the reaction medium inhibited proteolysis, back to a level similar to that observed against globin alone. Addition of heme did not change THAP activity towards ribonuclease, a non-hemeprotein substrate. The major storage protein of tick eggs, vitellin is a hemeprotein. Hydrolysis of vitellin by THAP is also inhibited by addition of heme to the incubation media. In a ligand blot assay, THAP is able to bind to either hemoglobin or vitellin being this binding totally inhibited by the addition of free heme to the reaction media. Taken together, our results suggest that THAP uses heme bound to protein substrates as a docking site to increase specificity and regulate VT degradation accordingly to heme availability.

Supported by: PADCT, CNPq, FINEP, FAPERJ, CAPES and PRONEX

VT-50 – TEST OF DIFFERENT PROTOCOLS AIMING AT THE MORPHOLOGICAL CHARACTERIZATION OF INTERNAL STRUCTURES OF EMBRYOS FROM THE TICK *BOOPHILUS MICROPLUS* AND NEOTROPICAL *ANOPHELES*

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Our final aim is to assist in the development of new control strategies of the hematophagous arthropod vectors *Boophilus microplus* and neotropical *Anopheles*. To accomplish this, knowledge of their biology, including the unraveling of morphological aspects of their embryonic development is a prerequisite. Here we describe attempts on the observation of embryonic internal structures at the optic microscopy level.

Previous attempts to analyze tick embryo morphology, by Transmission Electron Microscopy, have proved unsuccessful. Examination of resulting samples suggested lack of penetration of resin and fixative. We have then reasoned that one of the major problems to be overcome was the impermeability of tick eggs. The protocol classically used to dechorionate *Drosophila* eggs was tested over *Boophilus microplus* ones. Additionally, different fixatives were employed (glutaraldehyde, paraformaldehyde, Millonig) and results will be presented. To our surprise, the conventional histological processing utilized has rendered *B. microplus* eggs permeable to paraffin impregnation. Also, the internal embryo tick structures were well preserved.

Concerning mosquito eggs, dechoriation or, alternatively, permeabilization of eggshell layers is needed, since mosquito embryos are covered by a highly sclerotized chorion. We have tested different procedures leading to the withdrawal or weakening of *An. albiparvus* eggs: the dechoriation protocol classically used for *Drosophila* eggs, a sclerotization inhibitor, an egg clarification method and various combinations of these assays. After these treatments, *Anopheles* eggs were submitted to the conventional steps involved in paraffin impregnation. Comparison of all samples after sectioning and staining revealed preservation of internal structures in clarified embryos, treated or not with the sclerotization inhibitor.

We aim at utilizing the protocols here defined to characterize the morphogenesis of internal structures during the whole embryonic development of both ticks and *Anopheles* mosquitoes.

Supported by: UNDP / WORLD BANK / WHO, PAPES II Program (Fundação Oswaldo Cruz), USAMRU-B, FAPERJ

VT-51 – EFFECT OF DIFFERENT SCLEROTIZATION INHIBITORS OVER *ANOPHELES ALBITARSIS* EGG DARKENING AND HARDENING

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The analysis of *Anopheles* embryo development is one of the requirements towards the construction of genetically modified malaria vectors refractory to *Plasmodium*.

In order to collaborate with the neotropical *Anopheles* embryo characterization, some drugs are being tested, over *An. albitarsis* synchronized eggs, since oviposition. These drugs are intended to keep the eggs bright and permeable, since they inhibit some steps on the sclerotization enzymatic cascade. In the present work the influence of these drugs on both egg darkening and egg permeabilization are presented. The effects of the sclerotized inhibitors used on viability are shown elsewhere (Cardozo et al., 1999).

For the eggs darkening inhibition assays, different concentrations of a Dopa decarboxylase (DDC) inhibitor were used in association with an anti-oxidant. Control eggs start darkening from oviposition on and, in about 50-60 minutes all of them are black. On the other hand, experimental eggs don't darken completely for at least 270 minutes. In some cases, embryos can be observed inside the egg through the stereomicroscope, until larvae hatching. Other substances are also intended to be tested, like a Phenol oxidase inhibitor.

Both darkening and hardening of mosquito eggshell are seen as consequences of the sclerotization process. We then reasoned that the same drugs that are able to inhibit egg darkening would also restrain egg hardening and consequently, render the chorion permeable. For permeability assays, control (laid over water) and experimental (laid over sclerotization inhibitors) *An. albitarsis* eggs were put in contact with ethylene glycol (known as a cryopreservant) at different moments after egg laying. The shrinkage of the eggs was recorded at each five minutes for a total period of 1 hour. The results of these assays point to a shrinkage delay in experimental eggs in comparison to the controls. Shrunken eggs never attain their normal volume again. Even eggs at oviposition (control and experimental) seem to shrank when in contact with ethylene glycol. If these data are confirmed, the existence of another factors, besides sclerotization, influencing the egg permeability will have to be considered.

We aim to define permeabilization conditions sufficiently strong to enable the cryopreservation of *Anopheles* embryos.

Supported by: UNDP / WORLD BANK / WHO, PAPES II Program (FIOCRUZ), USAMRU-B.

VT-52 – THE SEARCH FOR ROSS CELLS IN THE *ANOPHELES AQUASALIS* MIDGUT: A POSSIBLE TARGET FOR *PLASMODIUM* INVASION

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Anopheles mosquitoes are the main vectors of malarial *Plasmodium* to the vertebrates. *Anopheles aquasalis* is a primary vector of human malaria in the coastal Brazil mainly in the North and Northeast regions. The parasite life cycle is initiated when the infected blood from the vertebrate is ingested by the female mosquito. The establishment of the infection occurs in the midgut during the bloodmeal digestion. The parasites need to interact and cross the epithelial cells of the midgut in order to produce oocysts. At the present this interaction process is not well understood. The main goal of this study is to recognize the morphology of the midgut epithelium and the Ross cells, the target for *Plasmodium* invasion in *Aedes aegypti* (Shahabuddin and Pimenta, 1998 PNAS-USA 95 3385-3389). This information will help further interaction studies. Mosquito midguts were dissected and divided in two parts: thoracic and abdominal regions, and processed routinely for transmission electron microscopy. The ultrastructural analysis of the thoracic midgut demonstrated a typical epithelium with the presence of a main cell population composed by columnar cells. The apical region of these cells are completely covered by microvilli, the cytoplasm is dense with a central nucleus and prominent nucleolus. In the basal region, the plasma membrane is folded in contact with a basal lamina. These muscle cells have variable shapes and are continuous with the epithelium basal lamina. The structural aspect of abdominal midgut is slightly different from the thoracic part. Besides the main epithelial cells, large cells projecting to the midgut lumen can be observed. The apical region of these cells is irregular with few microvilli and dense cytoplasm with no visible organelles. The aspect of this cell population is very similar to the Ross cells present in the *Ae. aegypti* midgut. This preliminary study is the basis for further analysis of the interaction process between the midgut and the malarial parasites, which could help provide essential information on how to block the infection in the mosquitoes.

Financial support: FIOCRUZ, CNPq, CAPES/PICDT/UFPA and PRONEX.

VT-53 – PARTIAL CHARACTERIZATION OF GLUTATHIONE S-TRANSFERASE AND ARGININE KINASE IN THE SALIVARY GLANDS OF THE MALARIA VECTOR, *ANOPHELES DARLINGI*

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Anopheles darlingi is the main vector of malaria in Brazil. Their salivary glands are directly involved in transmission of malaria parasites. The sporozoites, infective forms of *Plasmodium sp.* are located in the salivary glands before being inoculated in vertebrates hosts during mosquito feeding. We have investigated the expression of genes in the salivary glands of *Anopheles darlingi*. Two cDNAs were partially characterized. One of them, corresponds to a glutathione S-transferase (GST) cDNA, showing high similarity to a previously studied *Anopheles gambiae* GST. Glutathione S-transferase is a family of enzymes catalyzing the conjugation of reduced glutathione (GSH) to a wide variety of electrophilic compounds. In insects GSTs have been associated with insecticide resistance. The activity of GST in the salivary glands were detected by biochemical analysis utilizing like substrate the 1-chloro-2,4-dinitrobenzene (CDNB). The activity of GST in the salivary glands extract showed higher than in mosquito extract. The sequence of the second cDNA displays similarity to arginine kinase (AK) cDNAs. AK is a member of the phosphagen kinase, which reversibly catalyze the transfer of phosphate from phosphoguanidine to ADP, yielding ATP, in cells which have variable metabolic outputs such as muscle, neurons, transport epithelia, photoreceptors and spermatozoa. Antibodies against *Drosophila melanogaster* muscle AK were used to identify the protein in the salivary glands of *Anopheles darlingi*. The anti *Drosophila melanogaster* AK serum recognizes specifically a polypeptide with approximately 40 KDa in *Anopheles darlingi* salivary glands extract.

Supported by: FAPESP.

VT-54 – COMPARATIVE WESTERN BLOT ANALYSIS OF THE MIDGUTS OF MALARIA MOSQUITO VECTORS¹Macedo, E. M., ²Shahabuddin, M., ²Fields, I., ²Wang, P., ³Lima, J.B.P., ⁴Braga, E.M. & ¹Pimenta, P.F.P.¹Laboratório de Entomologia Médica, Centro de Pesquisas René Rachou – CPqRR Fundação Oswaldo Cruz – FIOCRUZ P.O. Box 1743. Av. Augusto de Lima 1715, Belo Horizonte – MG, ²Laboratory of Parasitic Diseases, NIAID-NIH, Bethesda, MD- USA and ³US Army Medical Research Center Unit, Instituto de Biologia do Exército, Rio de Janeiro, Brasil. Departamento of Parasitology, Universidade Federal de Minas Gerais, Belo Horizonte, MG., Brazil. (pimenta@netra.cpqrr.fiocruz.br)

The development of malarial *Plasmodium* in mosquitoes is related to several conditions involved in the interaction between the parasite and the vectors. The parasites are ingested with the bloodmeal and are initially located inside the mosquito midgut. Inside the midgut the parasites have to deal with temperature change, pH, peritrophic matrix and digestive enzymes until they can differentiate to ookinetes and become able to attach and cross the intestinal epithelium into the hemocel. The attachment of the parasites occur directly to the microvilli of the epithelial cells and the initial invasion throughout the Ross cells, as described by (Shahabuddin and Pimenta, 1998. PNAS-USA 95: 3385-3389). Preliminary studies of Altaf et. al. (Infection and Immunity 1994. 62: 316-318) have demonstrated that antibodies against the mosquito midgut disturb the invasion process and reduce the number of oocysts. In this study we used monoclonal antibodies produced against the *Aedes aegypti* midgut, to see the expression of similar components in the midguts of: *Anopheles gambiae*, *An. aquasalis* and *Ae. fluviatilis*; all of which are natural mosquito vectors of distinct species of *Plasmodium*. The initial binding of the antibodies to the midguts was seen using immunofluorescence assays. It was observed that the midguts of the all the studied mosquitoes bound the antibodies raised against *Ae. aegypti*. Midgut extracts from the same mosquitoes were submitted to SDS-PAGE and Western blot analysis. Comparative observation demonstrated that *Ae. fluviatilis* has a midgut protein pattern distinct from *Ae. aegypti* but similar to *An. gambiae* and *An. aquasalis*. The identification of these proteins and elucidation of their role in the interaction of the mosquitoes with *Plasmodium* will be attempted in future studies.

Financial support: FIOCRUZ, WHO, CNPq and PRONEX.

VT-55 – BLOOD MEAL INDUCED DIGESTIVE ENZYMES IN THE MIDGUT OF BRAZILIAN MALARIA VECTORS

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We have analyzed the presence of several digestive enzymes in the midgut of blood fed anopheline mosquitoes (*Anopheles darlingi*, *Anopheles aquasalis* and *Anopheles albitarsis*). The major digestive protease activity, trypsin, was found restricted to the posterior portion of the midgut. The trypsin activity increases rapidly after blood ingestion and attains a maximum between 18 and 24 hours after feeding. In the midgut of sugar fed mosquitoes, only about 7% of the maximum trypsin activity are found.

Aminopeptidase activity is also restricted to the posterior midgut, however presents a different induction profile after blood meal. The aminopeptidase activity starts to increase only at 3 hours after blood ingestion. The maximum activity of the aminopeptidase seems to be delayed compared with trypsin.

Different from the proteases, α -glucosidase activity is high in the midgut of sugar fed mosquitoes (about 50% of the maximum in blood insects). Just after blood ingestion, α -glucosidase activity in the anterior portion of the midgut decreases, indicating that the enzyme is carried to the posterior region with blood. The maximum level of α -glucosidase activity in blood fed mosquitoes is only 1.5 times higher than in sugar fed mosquitoes.

Our data indicate some differences on the pattern of digestive enzymes induction of Brazilian anophelines, when compared to the Africa and Asian mosquitoes (*Anopheles gambiae* and *Anopheles stephensi*). These differences could influence the capacity of Brazilian mosquitoes to transmit malaria, since the parasites have to survive and suffer modifications in the midgut lumen.

The study of gut specific genes coding for proteases and other enzymes appears as a good model not only because the use of a mosquito defective in proteases could lead to an interference with the parasite penetration through the peritrophic membrane, but also, and most important, because gut specific blood meal induced promoters are good candidates for driving the expression of molecules able to block the parasite invasion inside the insect.

Financial Support: FAPESP

VT-56 – EFFECTS ON THE VIABILITY OF ANOPHELES ALBITARSIS EGGS TREATED WITH SCLEROTIZATION INHIBITORS

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The construction of genetically modified *Anopheles* mosquitoes, refractory to the *Plasmodium*, appears as an alternative to malaria control. To attain this objective, the injection of exogenous DNA into eggs and the cryopreservation of embryos from different mosquito mutant and transgenic lines are some of the requirements to be achieved. In both cases a chorion permeabilization method is necessary, since mosquito eggshell is extremely sclerotized.

The action of some sclerotizing inhibitors on eggshell layers hardening and darkening has been tested (Jesus-Martins et al, 1999). However, it is also necessary to determine the role of these drugs on mosquito development, since reduction of viability could impair attempts of using these sclerotizing inhibitors on both exogenous DNA injection and embryo cryopreservation.

The effect on egg viability of different sclerotization inhibitors was tested since oviposition. In some cases several concentrations were used. The combined effect of sclerotization inhibitors and an antioxidant was also tested. Samples were analysed daily to record larvae hatching. We verified that, in the conditions established for an adequate hardening and darkening inhibition, eggs hatch at least as efficiently as controls.

We intend to test if the whole mosquito development is also normal when sclerotizing inhibitors are used during embryogenesis. This includes the efficacy of attaining adult stage, of mating and egg laying.

Supported by: UNDP / WORLD BANK / WHO, PAPES II Program (FIOCRUZ), USAMRU-B.

VT-57 – COMPARATIVE BIOCHEMICAL STUDY OF THE *AEDES FLUVIATILIS* MIDGUTS INFECTED OR NOT WITH *PLASMODIUM GALLINACEUM*

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The main goal of this study was to detect possible differences during the process of the bloodmeal digestion between *Aedes aegypti* mosquitoes, infected or not with *Plasmodium gallinaceum*. The mosquito midgut contents were submitted to SDS-PAGE and Western blot analysis. In order to process the experiments, mosquitoes were fed on normal chicken or on *P. gallinaceum* infected chicken. Three groups were analyzed: (1) mosquitoes fed on normal chicken; (2) mosquitoes fed on *P. gallinaceum* infected chicken; and (3) 15 day *P. gallinaceum* infected mosquitoes fed on normal chicken. The midguts were dissected at different times after the bloodmeal (immediately, 3h, 6h, 12h, 24h, 48h and 72h) and macerated in 0.05% Tween/PBS in the presence of protease inhibitors. The midgut extracts were submitted to 10% SDS-PAGE and analyzed. There were no visualized bands in the midgut extracts collected after 48h suggesting the total digestion of the bloodmeal after this time. Several protein bands (209, 182, 120, 68, 57 e 40 kDa) were visualized only on the groups 2 and 3, originated from infected mosquitoes or from mosquitoes fed on infected bloodmeal. Therefore, midgut extracts (3h after the bloodmeal) from the three mosquito groups were processed for Western blot analysis using antibodies against *P. gallinaceum* raised in mouse or obtained from the serum of infected chicken. A specific 120 kDa protein band was recognized by the mouse antibody and two bands, 73 and 81 kDa, recognized by the infected chicken serum. There was no detection of any protein bands in the midgut extracts from the group 3, the normal mosquitoes fed on the normal blood. Further studies are necessary to characterize these differences and to determine which proteins are those specific expressed during the blood digestion of the infected mosquitoes.

Financial support: FIOCRUZ, WHO, CNPq and PRONEX.

VT-58 – PARTIAL PURIFICATION OF *AEDES AEGYPTI* GUT CHITINASE

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The peritrophic matrix (PM) is a extracellular chitin-protein structure present in the midgut of the mosquitoes involving the food bolus. It is recognized as a potential physical barrier to parasites which develop and multiply within the midgut. The knowledge about which set of enzymes are involved in the PM synthesis and degradation is still unknown. The aim of this study is to purify and characterize the chitinases of the midgut of *A. aegypti* and correlate this enzyme with the formation and degradation of the PM. Measurement of chitinolytic activity in homogenates of *A. aegypti* midguts, dissected at different times after blood feeding, with the substrate 4-methylubelliferyl-D-N,N',N''-triacylchitotriose, showed that highest enzyme activity appeared between 40-48h after the blood meal. Approximately 300 midguts from 44-48h blood fed mosquitoes were homogenized in 0.1M Tris-HCl, pH 8.0 and centrifuged at 14000g for 10 min. The supernatant was applied to DEAE-Sepharose and two chitinase activities were separated. The highest activity, the unbound chitinase, was dialysed against 50mM acetate buffer, pH 5.5. After dialysis the sample was submitted to affinity chromatography using chitin as a matrix. The enzyme was eluted with 0.1N HCl, partially concentrated and loaded into a SDS/PAGE eletrophoresis. The eletrophoretic profile showed seven protein bands. We also identified the chitinase activity using a SDS/PAGE. After the eletrophoresis, the gel containing 0.01% glycol chitin was incubated in 0.1M acetate buffer, pH 5.0 with 1% Triton X-100. After incubation, the gel was transferred in a fresh solution of 0.01% Calcofluor in 0.5M Tris-HCl (pH 8.9). The chitinase band activity was visualized as a dark band in a fluorescent background under an UV transilluminator. Further purification steps are in progress.

Supported by CNPq and FENORTE.

VT-59 – CALIBRATION OF AN *Aedes aegypti* INSECTICIDE RESISTANCE DETECTION METHOD BASED ON THE USE OF IMPREGNATED BOTTLES (AS RECOMENDED BY CDC) IN ORDER TO COOPERATE WITH THE DENGUE CONTROL AT BRASIL

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Development of insecticide resistance is nowadays a serious problem, mainly among vectors of emerging diseases. Until 1992 it has been reported that 56 *Anopheles* and 39 *Culex* species presented some insecticide resistance, which interferes with vector control programs.

The World Health Organization recommends the use of impregnated papers as a standard evaluation method for insecticide resistance of adult mosquitoes (WHO, 1981). However, some difficulties associated with this protocol are: 1) this test has to be made with adults directly caught in the field, which poses problems concerning the age and the number of mosquitoes; 2) it is recommended that the test takes place with fed mosquitoes, which is not always possible in the laboratories; 3) results have to be recorded during 24 hours, and 4) few places in the world produce the impregnated papers and, consequently, their availability to the majority of the laboratories is very limited.

The Centers for Disease Control (CDC, Atlanta), has developed an alternative methodology to detect resistance to insecticides. This methodology is based on the use of 250 ml bottles impregnated with diluted insecticides. Some advantages of these assays are: 1) F1 mosquitoes reared in the laboratory are used, overcoming problems such as specimen number and age; 2) mosquitoes are submitted to the test without a blood meal; 3) recording of results takes only 2 hours, maximum and, 4) any technician can be trained in the bottle impregnation methodology.

We set up this alternative method in our laboratory using the susceptible *Aedes aegypti* Rock strain (provided by CDC) as reference lineage. The insecticides tested were malathion, fenitrothion and temephos. In our hands, 100% of mortality are attained at 30 minutes for malathion at 400 ug/bottle, 60 minutes for fenitrothion at 800 ug/bottle and 75 minutes for temephos at 900 ug/bottle.

Since we have both a reference susceptible *Aedes aegypti* strain and a calibrated protocol to record insecticide resistance, we intend to test this methodology on wild mosquito populations in order to assist vector control programs.

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VT-60 – PLANT EXTRACTS INDUCE STRUCTURAL MODIFICATIONS AND PROTEIN SYNTHESIS IN PERITROPHIC MATRIX OF *Aedes aegypti* LARVAE

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The gut of most of the insects contains a layer of acellular material containing chitin separating the food of the epithelial cells of the gut. This layer is called peritrophic matrix (PM). Our knowledge concerning this structure is still quite incomplete. However, there is increasingly interest for the study of this structure due to its probable function as a barrier against pathogens. The most obvious PM function is the mechanical protection of the intestinal epithelium against the damages caused by the abrasion of food particles. Peritrophic matrix of some insects also can carry out a secondary function as a barrier against the chemical attack of potentially toxic agents. The aim of our work is to test different crude alcoholic extracts of Leguminosae plants for larvicidal activity against *Aedes aegypti* larvae. We also ascertained alterations in the structure and protein constitution of PM caused by the extracts. We tested fourteen extracts of microorganism resistant plants. The extract of *Derris urucu* was the most active of all extracted tested. It promoted the death of 100% of the larvae at 250 ug/ml. The other toxic plants were *Dalbergia nigra*, *Mirocarpus fastigatus* and *Zollernia illicifolia*. The larvae submitted to the extract of these three plant species excreted long feces, being constituted mostly by entire PMs indicating these extracts probably contain a chitinase inhibitor. PMs, presents in the feces of larvae maintained in the presence of active extracts, were collected and its protein content was analyzed by SDS/PAGE. We observed that these extract seems to induce novel PM proteins. Second instar larvae maintained in the presence of 150ug/ml of the leaf extracts of *Myrocarpus fastigatus*, *Dalbergia nigra* and *Zollernia illicifolia* had their development retarded. By light microscopy, we observed that the structure of PM is strongly altered by these extracts. These extracts are been fractioned phytochemically to identify the active components responsible for the observed activity against *A. aegypti* larvae.

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VT-61 – IDENTIFICATION OF HEME BINDING PROTEINS IN PERITROPHIC MATRIX OF *Aedes Aegypti*

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An important event is observed in the adult females mosquitoes in response to the blood meal: the formation of an extracellular matrix containing chitin, separating ingested food from the gut epithelial cells. This layer is called peritrophic matrix (PM). Our goal is to investigate the PM physiological role as a chemical barrier to the potentially toxic heme released from ingested red cell hemoglobin. By spectrophotometry, we showed that the amount of heme stays practically constant in the gut during digestion. Histochemical studies revealed that around 12 hr after blood meal (abm) a discrete and uniform layer surrounding the blood bolus could be seen. At 14hr abm the PM was thicker and yellow and 24h abm the PM was completely formed with an intense yellow color. The ultrastructural observations confirmed the results described above showing electron-dense inclusions characteristic of heme deposition on the PM at 24hr abm. We could also observe the enormous net of microfibers of which PM is constituted and how complex it is at this time (24h). We also carried out 'in vitro' heme binding studies. Isolated dissected PM can bind nearly 5% of the heme present in the midgut. Several heme-binding protein bands were detected in polyacrilamide gels stained with dimethoxybenzidine. By Coomassie staining method, the eletrophoresis profile showed the presence of two predominant *A. aegypti* PM heme-binding proteins. Additional studies are being carrying out in order to purify, to characterize and to understand the physiological antioxidant role of these proteins.

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VT-62 – ANALYSIS OF SALT STRESS PROTEIN RESPONSE IN PERITROPHIC MATRIX OF *Aedes Aegypti* LARVAE

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The distribution of mosquitoes is largely based on the availability of suitable sites for larval growth. Because mosquitoes larvae are aquatic, water is essential. However, water sources vary considerably in composition. Mosquitoes larvae have developed physiological mechanisms that permit them to survive in extreme conditions in terms of salt composition. Mosquitoes like other hematophagous insects synthesize a peritrophic matrix containing chitin, protein, glycoprotein and proteoglycan that separate the ingested food from the gut. Proteoglycans and glycoproteins could play an important role in regulating the movement of water and ions across the PM. Depending of the mosquito life stages, the PM is formed from a secretion produced by midgut epithelial cells (PM I, in adults), or by specialized cells of the cardia, at the junction of the foregut and midgut (PM II, in larvae). It can be an important physical and chemical barrier in the insect gut. We decided to investigate a possible alteration of the protein composition of the *Aedes aegypti* PM after submission to salt stress. The PMs, present in fecal pellet, were collected and their protein content was analyzed by SDS-PAGE. We observed that PM proteins were induced in larvae maintained in concentrations of NaCl between 200 mM and 520 mM. Also, we observed the induction of novel PM proteins in larvae maintained in concentration of 260 mM of KCl and CaCl₂. The larvae when maintained under stress conditions produce great amount of feces. The first step of purification of these proteins was accomplished through a affinity chromatography using chitin as a matrix. With further characterization and aminoacid sequencing of these proteins it would be possible to identify their physiological role.

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