Vetores - Vectors

VE01 - Pathogen transmission by blood-sucking arthropods: an evaluation of the main concepts among high-school Brazilian students.

Leonardo Rodrigues Cunha, Alan B. Carneiro and Mário A. C. Silva-Neto.

Instituto de Bioquímica Médica, UFRJ, Rio de Janeiro, Brazil.

Chagas disease is caused by the trypanosomatid protozoon *Trypanosoma cruzi*. Such disease was described in the early twentieth century by Carlos Chagas, who reported the pathophysiological aspects and also its mechanism of transmission in a series of studies published in 1909. Those findings represented a unique achievement in the history of Medicine and have played a major role in the establishment of Brazilian biomedical research institutions. The present work was conducted in order to allow a diagnosis regarding the quality concepts taught on the transmission of infectious-disease transmission in high-school students in the state of Rio de Janeiro. Our research was focused on institutions of secondary education. We have evaluated such concepts through a questionnaire with objective questions and of big simplicity applied to 1700 students. Questions included knowledge regarding the ability of such students to identify and discriminate among the vectors, the pathogens and the mechanisms of transmission of: Chagas disease, Malaria and Dengue. Our main finding was that regular schools do not properly introduce the concepts of pathogen transmission by blood-sucking arthropods. The biggest deficiency was among students from the courses on general formation compared to specific medical or clinical courses. The lack of information is particularly severe concerning the identification of vector and pathogen of each disease. Regarding disease transmission the performance of students attending both medical and clinical courses was appropriate. However, the performance of the later group of students was below the levels required to students being prepared to work in public health. In conclusion, our data show that specific didactic and policies tools should be developed in order to fulfill the needs reported here and also to enhance the interest of Brazilian students on both vector and parasite biology while in high school.

Supported by FAPERJ.

VE02 - Vector Hematophagic Capacity is Suppressed by Parasite-Derived Glycoconjugates

Gazos-Lopes,F.; Mesquita, R.D.; Telhado, R.S.; Folly, E..; Machado, E.A.; Perales, J.; Romeiro, A.; Medonça-Previato, L.; Previato, J.O.; Atella, G.C.; Silva-Neto, M.A.C.

Rhodnius prolixus is a blood-sucking bug who depends on a series of antihemostatic molecules present in its saliva in order to successfully feed on blood. One of such compounds is a vasodilatatory gas named Nitric Oxide (NO). NO production is catalized by a Nitric Oxide Synthase (NOS) present in specialized salivary glands. It has been previously demonstrated that *Trypanosoma rangeli* infection impairs *R. prolixus* salivary gland NO generation (Garcia *et al*., 1994). The mechanisms involved in the regulation of NO production during both salivary gland development and infection have not been characterized to date. Using Western bloting techniques, immunohistochemical analysis and a NO fluorescent probe (DAF-2A diacetate, Molecular Probes), we have observed that *T. rangeli* reduces both *R. prolixus* NOS levels and NO generation. Parasite surface glycoinositolphospholipids (GIPLs) were administrated to uninfected bugs in order to determine its possible role on the manipulation of salivary gland NOS activity. Insects were injected with *T. rangeli* or *Phytomonas serpens*-derived GIPLs or with *T. cruzi* mucins. Both *T. rangeli*-derived GIPLs decreased salivary gland NADPH-diaphorase activity, NO production and NOS levels. However, no effect was observed when *P. serpens*-derived GIPLs or *T. cruzi* mucins were administrated to the insects. Proteomic analysis indicated that nitrophorin 1, a NO carrier protein, is also depleted in groups treated with *T. cruzi*-derived GIPLs. In correlation with these data, we have also observed that *T. rangeli* and *T.cruzi*-derived GIPLs reduced insect salivary antihemostatic activities and vector feeding competence. In addition to this, we have observed major changes in the protein phosphorylation profile of GIPL-treated groups. It is concluded that *T. rangeli* GIPLs mediate host NOS suppression and the reduction of antihemostatic activities during infection of salivary glands. Supported by CNPq, FAPERJ, IFS.

VE03 - PHLEBOTOMINE SAND FLIES AND NATURAL INFECTION BY LEISHMANIA IN THE GAFANHOTO PARK, DIVINÓPOLIS, MINAS GERAIS, BRAZIL

MARGONARI, C.S.[1,2], XAVIER, D.C.A.[1], PIMENTA, P.F.P.[2], SOARES, R.P.P.[2], SARAIVA, L.[3], MELO, M.N.[3], ANDRADE- FILHO, J.D.[2]

[1] Fundação Educacional de Divinópolis/ Universidade Estadual de Minas Gerais, Minas Gerais, Brasil; [2] Centro de Pesquisas René Rachou/ FIOCRUZ, Belo Horizonte, Minas Gerais, Brasil; [3] Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil.

The Gafanhoto Park is forest remaining in the urban area of Divinópolis. A little information is available on its fauna and flora. In the present work, we intended to determine the sand fly species occurring in the area. Captures with luminous traps (HP and Shannon) were performed during 1 year (10/ 2006 09/ 2007). 281 specimens of sand flies of the 17 species had been captured: *B. brumpti* 11.38%, *B. pintoi* 2.13%, *L. amarali* 0.35%, *L. aragaoi* 28.11%, *L. bacula* 0.35%, *L. braziliensis* 0.35%, *L. christenseni* 5.33%, *L. cortelezzi* 0.35%, *L. evandroi* 0.35%, *L. lenti* 0.71%, *L. lutziana* 17.43%, *L. monticola* 6.76%, *L. neivai* 4.62%, *L. sordellii* 2.84%, *L. teratodes* 0.35%, *L. termitophila* 1.06%, *L. whitmani* 9.6%, *Lutzomyia* sp. 8%. Highest densities of sand flies were often observed after rain occurrence. Using the Shannon trap, 68 individuals from 9 species were captured. From those, 47 females were tested using PCR for natural infection withr *Leishmania* spp: 7 *L. whitmani*, 1 *L. lenti*, 1 *L. lutziana*, 4 *L. neivai*, 1 *L. monticola*, 1 *L. christenseni*, 1 *L. pessoai* (34%), they had been positive for infection. Some products of PCR had been submitted to the clivagem with the enzyme of restriction Hae III: 1 *L. whitmani* presented natural infection for *L. chagasi*, 1 *L. monticola*., 1 *L. lutziana*, 1 *L. christenseni* and 1 *L. lenti* with *L. braziliensis*. These results demonstrated that there is a wild cycle with a high rate of *Leishmania* infection in the Gafanhoto Park. Those data reinforce the epidemiological surveillance in the area aiming to prevent Leishmaniasis outbreaks in Divinopolis.

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VE04 - INFECTION BY *TRYPANOSOMA RANGELI* AFFECTS MOULT INTERVAL, WEIGHT LOSS AND LONGEVITY IN *RHODNIUS PROLIXUS*

Ferreira, L.L.; Lorenzo, M.G.; Guarneri, A.A.

Centro de Pesquisas René Rachou, Fiocruz, Belo Horizonte, MG

T.rangeli is a protozoan parasite that shares hosts, mammals and triatomines, with *T.cruzi*, the etiological agent of Chagas disease. Although *T.rangeli* has been considered nonpathogenic to human hosts, it is able to produce different levels of pathogenicity in their invertebrate hosts. The effects of *T.rangeli* infection were evaluated on the duration of the fourth instar, the weight loss during the inter moult period and the longevity of *R.prolixus* adults. The duration of the fourth instar was evaluated in nymphs infected by injection into the thorax (10, 10^2 or 10^3 parasites) or orally by feeding on an artificial apparatus containing parasites (10^7 par/ml) and blood. Control groups were inoculated with PBS or fed on uninfected blood. Fifth instar nymphs inoculated by injection were fed on mice and weighed every three days for weight loss estimation. After moulting, the adults were maintained starved to evaluate their longevity under this condition. The comparison of the length of the fourth instar showed that the presence of the parasite on the haemolymph was sufficient to prolong that period (ANOVA; $p<0.05$), since in those insects that presented parasites only in the intestinal tract, the duration of the fourth instar was similar to control groups. The effect of infection on this parameter was dependent on the number of parasites inoculated, since the group that received 10 parasites was similar to control. Infected insects showed a lower daily weight loss than controls (T test, $p<0.001$). This reduction allowed infected insects to maintain a total weigh loss similar to control insects, even though they had a prolonged inter moult period. The infection prolonged the longevity of fastened adults (Kaplan-Meier, $p<0.05$). It is suggested that a reduction on the metabolism of infected insects could be responsible for the results observed. Additional assays will be necessary to test this hypothesis.

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VE05 - DEVELOPMENT OF *LEISHMANIA (LEISHMANIA) CHAGASI* (MHOM/BR/70BH46) IN *LUTZOMYIA (LUTZOMYIA) LONGIPALPIS* BY MEMBRANE FEEDING

FREITAS, V.C., PARREIRAS, K.P., SECUNDINO, N.F.C., PIMENTA, P.F.P.

Laboratório de Entomologia Médica, Centro de Pesquisas René Rachou- FIOCRUZ, Belo Horizonte, Minas Gerais, Brazil.

Studies on Leishmania-vector interactions constitute an important research field, since it may further knowledge on the many processes involved in the parasite transmission and thus Leishmaniases epidemiology. Lutzomyia longipalpis is the most important vector of Leishmania chagasi, the causative agent of American Visceral Leishmaniasis. Membrane feeding of hematophagous arthropods was first undertaken in 1912 to infect vectors with microorganisms in order to study their development and transmission. Based on the same methodology, sandflies have been experimentally infected with many species of Leishmania since 1927 to obtain known concentrations of microorganisms that can be put into feeding solutions. The aim of the present investigation was to study L. chagasi development in L. longipalpis by chick skin membrane feeding. Adult female sandflies were infected by feeding on mouse blood containing promastigotes ($4x10^7$/mL) at 37 ºC. Blood-engorged females were separated, maintained on 50% sucrose ad libidum at 25ºC and 95% air humidity and sacrificed at days 2 to 10 after blood feeding. L. chagasi developed well in L. longipalpis producing high infection rates. The percentage of fed sandflies was 48% (834/1741) and the infection rate was 87% (172/197). Noteworthy, the number of parasites per fly recorded at 48h ($2,4x10^4$) was lower than the initial concentration, which indicates that a proportion of parasites were killed in early infection. This value also decreased at day 3 after infection, probably due to bloodmeal digestion. Thereafter, the number of parasites per fly steadily increased to a peak at day 6 (approximately $2,4X10^4$ per fly), before decreasing again and remained at $1x10^4$ parasites per fly until day 10. Colonization of the stomodeal valve was observed in the majority of infected female. Metacyclic promastigotes represented about 8% of morphological stages found in smears from late-stage infections. Further experiments are ongoing for better understanding the infective process of L. longipalpis.

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Key words: Leishmania chagasi, Lutzomyia longipalpis, development, membrane feeding

VE06 - Colonization and experimental infection of potential vectors species of American Tegumentary Leishmaniasis (ATL) captured in PETAR, Iporanga municipality, São Paulo.

SOUZA, L. D[1]; ALVES, I. C[1,2] ; PAIVA, B. R[1,3];GALATI, E. A. B[4]; BUENO, E. M. F[4]; GOMES, C. M. C[5]. & MALAFRONTE, R.S[1,6].

1-Laboratório de Protozoologia, IMTSP/USP, 2-Laboratórios de Investigação Médica, LIM/49, 3-Instituto de Ciências Biomédicas/USP 4-Depto. de Epidemiologia, FSP/USP, 5-Depto. de Patologia, FMUSP, 6- Depto. de Doenças Infecciosas e Parasitárias, FMUSP.

Sand flies, diptera insects belonging to the Psychodidae family, are responsible for the transmission of *Leishmania* genus parasites. Some species are known vectors, while others are considered as such only on epidemiological evidences. Therefore more accurate studies are important to clarify these doubts and to conduct them the colonization of sand flies is necessary. There are suspicions that in the Region of the Vale do Ribeira, *Nyssomyia intermedia* and *Ny. neivai* are involved in the transmission of ATL (American Tegumentary Leishmaniasis). As the region has a tourist potential, with high visitation numbers, studies on these potential vectors were carried out. For this purpose, sand flies were captured in the city of Iporanga with two main purposes: establishment of a colony of the most frequent species in the region: *Nyssomyia intermedia* and *Ny. neivai* and determination of experimental leishmania infection in these sand flies, by means of PCR reaction. Preliminary results of this study have demonstrated a better adaptation of *Nyssomyia intermedia* in comparison to *Ny. neivai*. Experimental infection was carried out with hamsters infected by *L. (V.) braziliensis* (M17593). Results showed that *Ny. intermedia* supported the infection that was demonstrated either by dissection and PCR technique showing an infection rate by 1.5%. Supported by LIM/49 and FAPESP (05/58311-5).

VE07 - *AEDES AEGYPTI* EXPRESSING MICROPLUSIN AS AN EFFECTOR MOLECULE FOR *PLASMODIUM* SPOROZOITES

MACIEL, C.[1]; KOJIN, B. B.[1]; CAPURRO, M. L.[1]

[1]Universidade de São Paulo, São Paulo, Brazil

Transmission of malaria parasites by mosquito vectors is dependent on the successful development of *Plasmodium sp.* infective forms, particularly the sporozoites, which are the forms that enter the vertebrate host. The genetic manipulation of mosquito vectors has been a strategy for malaria control. Microplusin is a cysteine-rich antimicrobial peptide originally described as an hemolymph component of the cattle tick *Boophilus microplus* and was purified from its eggs as described [1]. *Aedes aegypti* mosquitoes infected with *Plasmodium gallinaceum* were microinjected with 0.5µl of 10 µM or 30 µM microplusin at day 7 after post-blood meal. Injections were performed into the thorax with a finely drawn calibrated glass microcapillary tube. After 24 hours, individual pairs of salivary glands were dissected, homogenized in 10 µl PBS, placed on a hemacytometer and the sporozoites counted using phase-contrast microscopy. Controls (injected with PBS) and microplusin-injected mosquitoes displayed 100% prevalence of sporozoite infections in their salivary glands, consistent with the high susceptibility of the RED *A. aegypti* strain to the avian parasite. Control mosquitoes developed high mean intensities of sporozoites infection (550 sporozoites per salivary glands pair), while in mosquitoes microinjected with microplusin at 10 µM and 30 µM, the number of sporozoites was reduced to 93 and 100 sporozoites per salivary glands pair, decreases corresponding to 83% and 80%, respectively. The results showed that microplusin is highly toxic to *P. gallinaceum* sporozoites in relatively low concentration, however, did not present toxic to the *A. aegypti* mosquito vectors. We obtain the transgenic strains, that integrate a quimeric gene that containing the promoter region of the *A. aegypti* vitelogenin gene, the *maltase-like- I* signal peptide of *A. aegypti* and microplusin coding sequence (p*Mos*[3xP3-EGFP-AeVg-Micro]). Two transgenic lines were established and we are performing the challenge experiments. Reference: [1] Fogaça *et al.*, 2004. *Dev & Comp Immunology* **28:** 191-200.

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VE08 - THE IMPACT OF ANTI-PARASITES GENES IN TRANSGENIC MOSQUITOES

Kojin, B. B. [1], Maciel, C. [1], da Silva, A. L. C. [1], Capurro, M. L.[1]

[1] Departamento de Parasitologia – ICB – Universidade de São Paulo

biburini@hotmail.com

The circumsporozoite protein is a surface molecule and shown to play an important role on sporozoite budding during oocyst differentiation and seems to be crucial for mosquito salivary gland and vertebrate liver tissue invasion. These predominant features of the protein have lead to its development as a malaria vaccine candidate. Previous studies has shown that CSP is important in determining the parasite selectivity for vector and host species and region I and II are involved in this recognition. The aim of this project is to express recombinant peptides encompassing the conserved Region I and II of the CSP from *Plasmodium gallinaceum, P. falciparum,* which are phylogenetically closer and *P. vivax,* a phylogenetically distant from the others, in *Aedes aegypti* mosquito hemolymph using a transient system dsSindbis virus and germline transformation of mosquitoes. We hypothesize that these recombinants peptides will compete with the CSP present in the surface of the parasites for the receptors in the salivary gland preventing its penetration in this organ. We are also studying another kind of molecules which interact directly with the parasites, a fragment of bovine α-hemoglobin (residues 33-61) (αHb_{33-61}), was previously isolated from the midgut contents of *B. microplus* (Fogaça et al., 1999). A synthetic peptide based on the sequence of αHb_{33-61} is active in micromolar concentration against Gram-positive bacteria and fungi. The other molecule is angiotensin II, a peptide with important physiological role in mammals, also presents antimicrobial action against *P. gallinaceum* sporozoites. Different concentrations of αHb_{33-61} and angiotensin II were assayed on *P. gallinaceum* sporozoites, showing that both peptides were effective in immobilizing the parasites. Propidium iodide staining of peptide treated sporozoites suggest that these peptides act through plasma membrane disruption. These peptides have already been cloned in plasmids used for transgenesis of mosquitoes and two transgenic mosquitoes lines were obtained.

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VE09 - HEMOCYTES OF *Aedes albopictus* (DIPTERA: CULICIDAE)

Araújo, H. R. C.[1]; Alves, L. C.[2]; Brayner, F. A.[2]; Pimenta, P.F.P.[1]

[1]Laboratory of Medical Entomology –René Rachou Institute / FIOCRUZ – Avenida Augusto de Lima 1715 – CEP 30190-002 - Belo Horizonte, MG, Brazil, e-mail: helena@cpqrr.fiocruz.br
[2]Department of Parasitology, Aggeu Magalhães Institute / FIOCRUZ

In insects, the immune system includes both humoral and cellular responses. Hemocytes are considered equivalent to vertebrate blood cells, since they are able to defend insects against pathogens, parasites and other foreign bodies. Recognition of hemocyte populations is an important tool to understand the vector-pathogen interactions. The Aedes aegypti hemocytes were recently classified into six types. However, far less is known about Ae. Albopictus hemocytes. Our goal in this study was to characterize Ae. albopictus hemocytes by morphology, lectin labeling and phagocytosis of foreign particles. Hemocytes were harvested from hemolymphs obtained by mosquito thorax perfusions with anticoagulant solution. The cells were spread in glass slides and processed for Giemsa staining to be analyzed by light microscopy. For Laser Scanning Microscopy, the hemocytes were fixed and processed for labeling with FITC-fluorescent lectins. Phagocytosis assays were conducted by injecting FITC-latex beads into the mosquito thoraxes followed by hemolymph collection 30 min later. Our results showed that Ae. Albopictus hemocyte population is composed by six cell types varying in size and morphology. Similarly to the Ae. aegypti hemocytes, they were classified as prohemocytes, adipohemocytes, granulocytes, plasmatocytes, oenocytoids, and thrombocytoids. These hemocytes expressed ligands to ConA, WGA, BS1 and HPA lectins with distinct intensities of labelings, but two used lectins (PNA and LPL) did not bind in any hemocytes. The fluorescent lectin labeling did not discriminate the individuals in the hemocyte population. Besides, the phagocytosy probe showed that only granulocytes and plasmatocytes ingest latex beads. The present study is the first to describe the Ae. Albopictus hemocytes. Additional experiments are being developed to better identify these hemocytes and their phagocytosis processes. Such knowledge will contribute to better understand the cellular responses this mosquito vectors against pathogens.

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VE10 - Immune activation in *Aedes aegypti* by blood feeding and virus infection

Nascimento_Silva, M.C.L.; Oliveira, J. H. M.; Pereira, L.O.R.; Oliveira, P.L.; Sorgine, M.H.F.

Instituto de Bioquímica Médica, UFRJ, Brasil

Introdução e objetivos:

Diseases caused by arthropod-born virus are significant health problems, and novel control methods are required to block pathogen transmission. Although *Aedes aegypti* is a vector of important arbovirus, little is known about mosquito immune responses to virus. In this mosquito, infections by fungi are known activators of the toll pathway, through NF-Kb related transcription factor Rel 1, while bacteria mostly activate the IMD pathway, that operates through a different NF-Kb othologous protein, Rel 2. The JAK/STAT pathway is also related to pathogen clearance, a process that occurs by the regulation of complement-like humoral proteins.

Resultados e Conclusões:

In this work we observed that three major mosquito immune-related transcription factors (aaREL 1, aaREL 2 and STAT) are up regulated 24 hours after blood meal but not after albumin (BSA) or latex feeding. Additionally, mosquitoes artificially infected with sindbis virus didn't present any significant increase in the expression of these genes, when measured by real time PCR.

Mosquitoes treated four days with antibiotics before infection showed a reduced expression of REL 2, but not of REL 1 and STAT mRNA levels. Interestingly, sindbis infected mosquitoes pre-treated with antibiotics, as well as mosquitoes infected through an artificial latex meal, instead of blood, showed an increase in the viral load four days after infection when compared with mosquitoes infected with a normal blood meal.

These results suggest that the blood meal *per se* is able to activate innate immune pathways and the upregulation of REL 2 seems to be mediated by the gut microbiota, profoundly affecting viral loads in *Aedes aegypti.*

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VE11 - Characterization of the complement system inhibition promoted by the hematophagous insects *Triatoma brasiliensis*, *Triatoma infestans*, *Rhodnius prolixus* and *Aedes aegypti*

Barros, V.C.[1,2]; Assumpção, J.G.[2]; Cadete, A.M.[2]; Araújo, R.N.[2]; Pereira, M.H.[2]; Gontijo, N.F.[2]

[1]Departamento de Parasitologia e Microbiologia, CCS, Universidade Federal do Piauí, Teresina, PI. [2]Laboratório de Fisiologia de Insetos Hematófagos, Departamento de Parasitologia, ICB, Universidade Federal de Minas Gerais, Belo Horizonte, MG.

Haematophagous insects have salivary and/or intestinal inhibitors of the complement cascade which are involved with epithelial protection against the attack of the complement system. In order to better characterize the points of the complement cascade interrupted by these inhibitors, an immunological method was developed which was capable to determine the level of deposition of some complement factors on the activator surface disposed inside microplate wells in the presence or absence of inhibitors. Saliva, soluble intestinal content and intestinal membrane-bound-molecules were searched for inhibitory activity and used for characterization of inhibition. The intestinal content from the 3 triatomine species studied was able to inhibit the deposition of C4b and C3b by the classical pathway, and C3b by the alternative. The saliva from the 3 species was able to interrupt the deposition of the same factors except from *T. infestans* and *R. prolixus* which was ineffective to impair C4b deposition by the classical pathway. C1q deposition by the classical pathway was not affected in any condition studied. Intestinal membrane-bound-proteins were only investigated concerning their capability to interfere with C3b deposition by the classical pathway. In this case, materials extracted from the 3 species of triatomine bugs were unable to inhibit C3b deposition. The soluble intestinal content of *A. aegypti* acted as an inhibitor, impairing C3b deposition by both, the classical and alternative pathways. The interruption of the complement cascade in its activation steps makes the inhibitory activities more efficient in protecting the midgut of haematophagous insects. Supported by FAPEMIG, CAPES and CNPq

VE12 - COMPARISON OF ANTIBODY LEVELS TO SALIVARY ANTIGENS OF *LUTZOMYIA LONGIPALPIS* (DIPTERA: PSYCHODIDAE) IN SERA OF CHICKENS AND DOGS IN AN URBAN FOCUS OF VISCERAL LEISHMANIASIS

MENDES-SOUSA, A.F[1], MARTINS, K.A[1], ALEXANDER, B[2], BATES, P.A[2], CAVALCANTE, R.R[1].

[1]Universidade Federal do Piauí, Piauí, Brazil; [2]Liverpool School of Tropical Medicine, Liverpool, England.

Although chicken-rearing is commonly cited as a risk factor for humans acquiring visceral leishmaniasis (VL) in several Brazilian cities, the role of chickens in transmission of *Leishmania infantum* by the phlebotomine sand fly vector (*Lutzomyia longipalpis s.l.*) has not been resolved. Chickens are abundant in the marginal neighbourhoods in which most VL occurs and sand flies bite the birds avidly. However determination of the relative importance of chickens (which cannot sustain *Leishmania* infections) and domestic dogs (the most important host) are hampered by difficulties in obtaining insects with fresh blood meals for analysis. An alternative approach is to compare levels of antibodies to sand fly salivary antigens in the serum of different host species. In the present study serum samples were obtained from chickens and dogs in three poor neighbourhoods of Teresina (Piaui) an endemic focus of urban VL since the 1980s. These samples were analysed by ELISA, using antigen obtained by dissecting the salivary glands of laboratory-reared *Lu. longipalpis*. Of 493 samples from chickens tested to date, 388 (78.7%) were positive for antibodies to sand fly salivary antigens, approximately the same as the rate in samples from dogs (74/91 or 81.3%). Mean values for serum antibodies were 0,971 in chickens and 0,471 in dogs. These values confirm the importance of chickens as bloodmeal sources of *Lu. longipalpis* and suggest that at certain densities the birds could act as zooprophylatic agents for transmission of *Le. Infantum;*

VE13 - DETECTION OF IGY AGAINST SALIVA OF *LUTZOMYIA LONGIPALPIS* IN EGGS OF DOMESTIC CHICKENSBY ELISA

DIAS, L.R[1], B. ALEXANDER[2], BATISTA, E.K.F.[1], PAUL, A. BATES[2], CAVALCANTE, R.R.[1]

[1]Universidade Federal do Piauí;
[2]Liverpool School Tropical Medicine.

The principal avian immunoglobulin is know as IgY because of the high concentrations present in egg yolk is otherwise similar to mammalian IgG. In birds IgY produced in the serum is transferred to egg yolk, presumably including that produced as a result of biting by bloodsucking insects. Chickens are important sources of blood meals for phlebotomine sand flies, the vectors of *Leishmania infantum* in urban foci of visceral leishmaniasis (VL) in Brazil. Attempts to determine the relative importance of chickens and other hosts are hampered by difficulties in encountering insects with fresh blood meals and a possible alternative is to compare antibody levels to sand fly salivary antigens in serum. Since chickens concentrate IgY in yolk, it should also be possible to use eggs to estimate sand fly biting intensity on chickens and infection rates in the insects. Eggs were collected from three VL-endemic neighbourhoods of Teresina (Piaui). Yolks were separated, washed and measured before adding polyethylene glycol (PEG 6000) and PBS to remove impurities and the lipid fraction. The remaining fraction was retreated with PEG and PBS followed by centrifugation to precipitate IgY. Levels of antibodies to the salivary antigens of *Lu. Longipalpis* were then measured by ELISA. All but one of the 27 samples tested to date were positive for salivary antigens (ELISA: 450 nm; Positive Control: 0,584: negative Control: 0,105; σ^2: 0,92; s: 0,96). Levels were similar to those observed in serum from the chickens that laid these eggs, suggesting that harvesting of eggs provides a practical, accurate and less invasive method of estimating sand fly biting intensity on chickens. We are also investigating antibodies to *Leishmania* in chicken yolk as an indirect measure of infectionrate in the insect vector.

VE14 - Salivary and intestinal inhibitors protects the intestinal tract of the hematophagous triatomine *Triatoma brasiliensis* against the complement system

Barros, V. C.[1,2]; Cadete, A. M.[2]; Assumpção, J. G.[2]; Araújo, R. N.[2]; Pereira, M. H.[2]; Gontijo, N. F.[2]

[1]Departamento de Parasitologia e Microbiologia, CCS, Universidade Federal do Piauí, Teresina, PI.
[2]Laboratório de Fisiologia de Insetos Hematófagos, Departamento de Parasitologia, ICB, Universidade Federal de Minas Gerais, Belo Horizonte, MG.

According to our hypothesis one of the physiological roles of the complement inhibitors encountered in the triatomine saliva and intestinal content is to protect the anterior midgut (crop) against the injurious action of the complement proteins normally present in the ingested blood. To obtain evidences in favor of this hypothesis, 4° instar nymph(s) of *T. brasiliensis* were forced to ingest 50µL of 2x concentrated normal human serum (2x NHS), 50µL of inactivated 2x concentrated normal human serum (2x INHS), 50µL of 1x concentrated normal human serum (1x NHS) or take a normal blood meal in a human volunteer (NBM). After one hour, the nymphs were dissected and the crop treated with anti-human C5-C9 antibody coupled with fluorescein in order to observe the formation of the membrane attack complex (MAC) in the epithelial surface of the crop. The method employed for the forced ingestion of serum impairs the concomitant ingestion of saliva, so the insects submitted to this treatment had only the intestinal inhibitors to protect the midgut against the serum. The insects treated with 2x NHS were strongly marked, indicating lesions throughout the epithelium. The ones treated with 1x NHS were slightly marked while the insects treated with 2x INSH as well as that which had ingested a NBM were not affected. The fluorescent dye propidium iodide was used to access cell death in insects treated with 2x NHS and 2x INHS. As expected, the results showed a strong fluorescence in the insects treated with 2x NHS but not with 2x INHS. These results are strong evidences that the salivary and intestinal complement inhibitors are produced to protect the digestive tract against the complement system. Supported by FAPEMIG, CAPES and CNPq

VE15 - STUDY OF MOSQUITO IMMUNE-RELATED GENES AGAINST *PLASMODIUM* SSP.

Oliveira, S. B. [1], *Ibraim I. C.*[1], *Brito C. F. A*[1], *Ruiz J. C.*[2], *Moreira L. A.*[1]

[1] *Laboratório de Malária, Centro de Pesquisas René Rachou – FIOCRUZ, Belo Horizonte, Brazil;* [2] *Laboratório de Parasitologia Celular e Molecular, Centro de Pesquisas René Rachou – FIOCRUZ, Belo Horizonte, Brazil.*

Anopheline mosquitoes are the vectors of human malaria parasites and have developed efficient immune responses against *Plasmodium* spp.. The mosquito's immune system presents an essential factor towards the susceptibility or refractoriness to plasmodia, being an important tool for developing alternative approaches for malaria control. For instance, by finding new candidate target genes either for transgenic approach or for transmission blocking vaccines, or by the development of chemical components to block the interaction between the *Plasmodium* and the anopheline mosquito. Many anti-malaria immune genes have already been studied in *A. gambiae*. FBN9 and TEPs (Thioester Containing Proteins) have been described as important immune genes, specially against *Plasmodium* spp. infection. The main vectors in Brazil are *A. darlingi*, *A. aquasalis* and some species from the *A. albitarsis* complex. Since most mosquito immunity research focus on malaria vectors which are not present in Brazil, this project aims to study the immune-related genes FBN9 and TEPs from Brazilian mosquitoes and analyze the conservation between these species. The orthologs genes FBN9, TEP1 and a different TEP from *A. aquasalis* were identified by PCR and sequenced. We compared the obtained sequence with the *A. gambiae* protein sequence and found 77% of similarity for FBN9, 60% for TEP1 and 81% for the other TEP. The high similarity could suggest the same function between the orthologs. We also identified, by PCR, the genes FBN9 and TEP1 in *A. darlingi*, *A. albitarsis*, *A. brasiliensis* and *A. nuneztovari*. Currently we are sequencing these genes for a better understanding about the immune genes evolution in Brazilian vectors.

Support: CPqRR/ FIOCRUZ; FAPEMIG ; INPA; CPqL&MD

VE16 - NO production in murine macrophages is supressed by vector-derived saliva.

Senna, R.; Cunha, L. R.; Atalah, M.; Carneiro, A. B.; Gazos-Lopes, F,; Atella, G.C.; Silva-Neto, M. A. C.

Instituto de Bioquímica Médica, UFRJ, Rio de Janeiro, Rj, Brazil.

Rhodnius prolixus is a vector of *Trypanosoma cruzi* in South America. This parasite is transmitted by vector feces deposited on human skin during blood feeding. One of the routes of host cell invasion occurs through the wound produced by the insect bite. *R. prolixus* saliva and feces are sources of the bioactive lipid lysophosphatidylcholine (LPC), which is a powerful modulator of cell signaling in mammalian cells. The purpose of this study was to characterize the effects of *Rhodnius prolixus* saliva and LPC on NO production. The expression of inducible nitric oxide synthase (iNOS) gene in lipopolysaccharide (LPS) stimulated murine macrophages was 100 % blocked by LPC as evaluated by western blotting. 1000-fold diluted saliva and LPC (10-150 μM) were able to reduce up to 100 % LPS-induced NO production in macrophages in a dose dependent fashion. We have previously shown that exposition to saliva manipulates the intracellular signaling system of host macrophages. Suppression of NO production does not rely on PI-3 kinase and MAP kinase activity since the inclusion of these protein kinases inhibitors (2 Ki) did not alter the effect of LPC. *T. cruzi* will meet a cell environment within the wound previously stimulated by saliva which might help parasite infection. The effects of both saliva and LPC on the gene expression profile will be followed by 2D-electrophoresis. Thus the mapping of the early intracellular signaling events triggered by saliva and LPC on murine macrophages may shed light on novel targets to block parasite transmission.

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VE17 - PHYLOGENETIC RELATIONSHIPS AMONG EIGHT SPECIES OF *CULEX* (DIPTERA: CULICIDAE) USING DNA SEQUENCE OF THE MITOCHONDRIAL GENE CYTOCHROME C OXIDASE I

SILVA, B. D.[1] , VESGUEIRO, F. T.[1], SALLUM M. A. M[1], MARRELLI, M. T.[1]

[1]Departamento de Epidemiologia, Faculdade de Saúde Pública, Universidade de São Paulo, São Paulo, Brazil

The genus *Culex* (Diptera: Culicidae) comprises approximately 763 valid species. It is one of the largest genera of Culicidae, but, it is one of the least known group. Identification of *Culex* mosquitoes based on morphological characteristics of the adult female can be difficult because many species belong to complexes, often with contrasted ecology, behavior and vector importance. In the present study the mitochondrial cytochrome oxidase I (COI) gene was examined for eight species belonging of three subgenera of *Culex,* with the objective of establishing phylogenetic relationship among these species and their subgenera. The phylogenetic relationships of *Cx. (Microculex) imitator, Cx. (Culex) dolosus, Cx. (Culex) coronator, Cx. (Culex) nigripalpus, Cx. (Culex) molllis, Cx. (Culex) bidens, Cx. (Melanoconion) zeteki* and *Cx. (Melanoconion) aliciae,* was inferred using the distance methods, the Neighbor Joining algorithm, under the Kimura 2-parameter model implemented in MEGA 4.0 software. Support for the groups was evaluated using distance bootstrap analyses with 500 replicates. The tree was rooted using the COI sequence of *Anopheles nuneztovari*. Results of the preliminary analyses showed that the sequences of species of the subgenera *Culex, Microculex* and *Melanoconion* clustered together in three distinct groups, agreeing with the morphological data. *Cx. (Cx.) dolosus* l.s. of two different localities showed high intra-specific variability, similar to two specimens identified as *Cx. (Mcx) imitator* l.s, corroborating morphological differences observed in male genitalia characteristics. Results of our analyses suggest that *Cx, imitator* and *Cx. dolosus* may comprise two distinct species. Using the COI sequence as a species barcode was suggested as an additional data for species identification and to solve taxonomic problems. Supported by FAPESP 05/50225-2 (MTM) and 05/53973-0 (MAMS).

Key words: *Culex,* mtDNA, COI, Molecular Taxonomy

VE18 - ANALYSIS OF THE SECOND INTERNAL TRANSCRIBED SPACER OF THE RIBOSSOMAL DNA FROM BRAZILIAN *CULEX* (DIPTERA: CULICIDAE) MOSQUITOES

VESGUEIRO, F. T.[1], SILVA, B. D.[1], SALLUM M. A. M[1], MARRELLI, M. T.[1]

[1]Departamento de Epidemiologia, Faculdade de Saúde Pública, Universidade de São Paulo, São Paulo, Brazil

The genus *Culex* is one of the largest genera of Culicidae and include vectors of several arboviruses and filarial worms. Despite that, the knowledge on its taxonomy and phylogeny is still scarce. The identification of the mosquitoes is generally made on the basis of morphological characters. However, many species of *Culex* are morphologically similar, making it difficult the correct identification. Currently, molecular techniques have been used in both evolution studies and species distinction, including those that belong to complexes. In this study, *Cx.* (*Cux*.) *bidens*, *Cx*. (*Cux.*) *chidesteri*, *Cx.* (*Cux*.) *coronator*, *Cx*. (*Cux*.) *dolosus, Cx*. (*Cux.*) *mollis*, *Cx*. (*Mcx*.) *imitator*, *Cx*. (*Mel*.) *zeteki* and *Cx.* (*Mel*.) *aliciae*, collected in several localities in Brazil, were identified based on morphological characteristics of the immature and adults. Sequences of the ITS2 of the rDNA of these species were generated to verify the level of inter-specific differentiation and their relationship with the morphological characteristics. A preliminary analysis was made on the basis of the size of this spacer after electrophoresis and visualization of the products. No differentiation in the size of the fragments among the species of the subgenera *Melanoconion* and *Microculex* was found, showing bands of the shortest length (~350bp). The species of the *Culex* subgenus showed the most variable and largest sizes (from ~450bp to ~500bp), with the largest ITS2 of *Cx.* (*Cux.*) *coronator*. The direct sequencing of the PCR products generated in most cases ambiguous data, indicating intra-individual variation. In this regards, the next step will be to clone the ITS2 PCR products to verify the degree of intra-genomic variation and the possibility of using the ITS2 sequence data for evolutionary studies on the Neotropical *Culex* subgenera. Supported by FAPESP (05/50225-2 - MTM, 05/53973-0 - MAMS) and CAPES.

Key-word: *Culex*, DNAr, ITS2, Molecular Taxonomy.

VE19 - Populational evolutionary patterns of the vector *Culex quinquefasciatus* in State of São Paulo

MORATORE, C.; SUESDEK, L.

Seção Parasitologia, Instituto Butantan, São Paulo, Brazil

The dipteran *Culex quinquefasciatus* is the main vector of bancroftian filariasis and arboviruses. Owing to its medical relevance it has been the target of several populational control attempts, which are considerably jeopardized by microevolution of mosquitoes**.** In State of São Paulo, two localities of occurrence of *Cx. quinquefasciatus* require particular epidemiological attention: A) Parque Ecológico do Tietê (PET) in São Paulo city, a touristic park inhabited by several vertebrates which are potential hosts of arboviruses. B) Pariquera-Açu (150 km apart from PET) a semi-rural municipality placed in the context of Atlantic forest. Hitherto, it was not known if there is genetical-morphological differentiation or gene flow between these populations from these geographically and ecologically distinct localities. Our aim was to identify occasional populational divergencies that could be indicative of microevolution. Biological parameters such as morphometrics and molecular studies were applied in order to compare the geographically distant populations (PET/Pariquera) living under different ecological conditions. By means of analysis of centroid size it was observed differences between wing sizes of both populations. Analysis of canonical variables also pointed to unequivocal dissimilarities among samples. The observed sexual dimorphism was statistically significant whereas bilateral asymmetry was not significant. Studies of rDNA variability showed equivalent endonuclease restriction patterns between populations. Micrography of eggs evidenced meristic and morphometrics chorion characters. When compared, they pointed to similarity between both populational samples. Molecular and egg parameters have not pointed to divergencies between populations, suggesting the possibility of existence of gene flow until recent times. However, wing morphometric characters exhibited geographical variation, which could be explained by the occurrence of microevolutionary events or plasticity of non-evolutionary nature. The next step is to evaluate the evolutionary significance of such variation which could in a future, enhance our knowledge on vector populational dynamics.

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VE20 - POLYMORPHISMS AND RAPID MORPHOLOGICAL VARIATION IN AEDES SCAPULARIS.

DEVICARI, M.; SUESDEK, L.

Seção Parasitologia, Instituto Butantan, São Paulo, Brazil.

Aedes scapularis (Diptera: Culicidae) is widely distributed in America, has plasticity to explore natural and urban environments. It bears vectoring ability for diverse arboviruses and it was responsible for Rocio virus transmission during the years 1970s. The previous knowledge on such species is very scarce and it is not known if there is geographic genetic-morphological polymorphism among populations. Moreover, data on the evolutionary significance of possible variation are not available, although it would be very helpful for development of controlling methods for this insect. In São Paulo State, two localities of occurrence of Ae. scapularis draw medical-epidemiological attention: the municipality of Pariquera-Açu (PAR) and city of São Paulo (SAO). We aimed to investigate possible populational variations in Ae. scapularis from these two localities SAO and PAR. Survey was perfomed by comparison of wing morphometrics and eggs micrography. Geometric morphometrics analyses showed sexual dimorphism of wing shape in SAO and PAR, and of wing size in SAO. In both the sexes the bilateral asymmetry was tenuous for shape and not significant for size. Interpopulational comparisons, SAO and PAR wings were distinguished by size and shape (between females) and by shape (males). PAR showed a temporal variation of wing shape within 8 months which was not detectable in size. MEV micrography of populational samples showed that PAR eggs are proportionally longer and with less conspicuous micropyle than SAO. In fact, there is a geographic variation between these two populations of Ae. scapularis since the samples can be unequivocally distinguished. Furthermore, wing size can suffer significant alterations in periods of time as short as 8 months. Present results will be possibly helpful for accurate identification of mosquitoes from doubtful provenience. Results also suggest that Ae. scapularis may comprise two or more cryptic species, hypothesis which will be tested in a near future.

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VE21 - Evidence of low gene flow among populations of Aedes aegypti *in São Paulo city.*

CARVALHO, E. & SUESDEK, L.

Seção de Parasitologia, Instituto Butantan, Brasil.

Introduction. The mosquito *Aedes aegypti* is among the most medically important insects. It is able to transmit yellow fever, dengue and occasionally other parasites. Dispersional dynamics of this species have been frequently addressed, since it is a central question in medical entomology. Objective. We aimed to determine the degree of similarity among these populations based on wing morphometric characters. On a semi-quantitative basis, we also intended to estimate the level o gene flow among them, if directly proportional to wings similarity. Methods. Populations of Ae. aegypti were collected in three localities in the expanded metropolitan area of São Paulo city: Vila Mariana (VM), Osasco (OS) and Butantã (BU). Linear distances between these localities ranged from 7 to 15 km. Methods of geometric morphometrics (GM) were applied, and, after digitalization of 18 wing landmarks, wing conformational consensus, centroid size and canonical variables were computed. Two morphometric analyses were done in order to compare the populational samples VM, OS and BU. Results. Populational samples were distinct for the morphometric characters analyzed. Centroid sizes were significantly different among them in pairwise comparisons (T-test, $p<0.05$) and were ordered: OS<VM<BU. Cannonical variables obtained from the multivariate comparison between each pair of populations indicated clear separation among samples. Mahalanobis distances among samples revealed that the minor distance is between the pair VM-OS whereas the major distance is VM-BU. Discussion. Present results demonstrate that these three populations are morphologically distinct indeed, which leads us to presume that they are not connected by a strong gene flow. The different female wing sizes deserve special attention since wing size it is arguably related to vectorial capacity. Nevertheless, it is known that somehow non-genetic plasticity of wing characters could also explain present results. To answer such conjecture, heritability of these morphometric characters must be tested.

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VE22 - New characters for taxonomic diagnosis of Culicids of medical importance (Diptera).

SUESDEK, L.

Seção de Parasitologia, Instituto Butantan, Brasil.

Introduction: The culicids Aedes aegypti, Aedes scapularis and Culex quinquefasciatus are dipterans of great interest for public health owing to the fact they are vectors of several human parasitoses. The correct taxonomic identification of a *species of medical interest* is very important. However, its identification is unfeasible when samples are stored in ethanol, since important taxonomic characters may be damaged by that fixative. Thus, characters which can resist to the fixation procedure should be useful in taxonomic approaches. Objective: We aimed to find taxonomic markers for Culicidae which could be used in cases that the sample is preserved in ethanol or even if the specimen is partially damaged. For that purpose, analyses of geometric morphometrics (GM) were applied. Methods: Specimens of Aedes scapularis, Aedes aegypti and Culex quinquefasciatus were fixed in ethanol 70%. The wings of males and females were mounted in slide-coverslip and digitally photographed. Methods of geometric morphometrics were applied, and, after digitalization of 18 wing landmarks, the following parameters were computed: wing conformational consensus, centroid size and canonical variables. Results: Data showed that when analysed together, wing consensus, centroid size and canonical variables had enough power to discriminate the three species among them. Cannonical variables analysed separately also exhibited adequate resolution for species identification. Sexual dimorphism was also evidenced by all parameters in these species. Discussion: We conclude that GM can have great taxonomical utility in evidencing species-specific wing characters in the three species. From this point on, with GM these species may be identified from either male/female ethanol-preserved individuals or even when only wings are available. We do believe that such approach can be extended to other species of public health importance.

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VE23 - EVOLUTION OF CULEX QUINQUEFASCIATUS: TEMPORAL AND GEOGRAPHICAL VARIATION, PRESENCE AND ABSENCE OF INSECTICIDE AND POLLUTION

PERUZIN, M. C. J.; SUESDEK, L.

Seção Parasitologia, Instituto Butantan, São Paulo, Brazil

The mosquito Culex quinquefasciatus has medical importance due to its ability of vectoring arboviruses and filariases. Microevolution in insecticide resistance is a remarkable limiting factor for populational control of this species. In the State of São Paulo there are two populations under different environmental conditions. One, near Pinheiros River (PIN), survives to pollution and pyrethroids applications and another, in Pariquera-Açu (PAR), lives in a semi-rural place in the absence of pollution and insecticides. The objective of this work was to investigate if these populations from different environments have genetic-morphological polymorphisms and if PIN population exhibits non-noticeable morphological variations along the time. Parameters used in the comparisons were ribosomal DNA (rDNA) and wing geometric morphometrics. The geometric morphometrics of 286 wings of PIN, collected in 2004 (PIN-04) and 2007 (PIN-07), and 150 wings of PAR, collected in 2008, demonstrates morphological variations. The two populations PIN-04 and PIN-07 revealed strong intrapopulational sexual dimorphism concerning shape and size, being the wings of females larger than those of males in both populations. The wing asymmetry is non-significant for size and tenuous for shape, being slightly larger in males and in PIN-07. The specimens of PIN-07 are larger and more bilaterally asymmetric than PAR, possibly due to higher food availability and to continuous exposition to high level of insecticide, respectively. Analysis of rDNA revealed restriction patterns equivalent for PIN-07 and PAR populations. Thus, one may suppose that gene flow may have occurred until recently. This study showed that it is possible to occur size and shape variation of wings in Culicidae in time intervals as short as three years. The next step would be to evaluate in depth the relationship between geographical-temporal variation and it possible causes like pollution and insecticides.

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VE24 - High phenotypic variability was detected in a possible hybrid zone of the *Triatoma brasiliensis* species complex, in the state of Pernambuco, Brazil (Hemiptera, Heteroptera, Reduviidae).

V.L. Neiva[1], M. Gumiel[1], G. Oliveira[2], M.M. Lima[1], E. Dotson[3], P. Cabello[1], C.E. Almeida[1], J Costa[1]

1- Instituto Oswaldo cruz, FIOCRUZ, Av. Brasil 4365, RJ, Brasil.
2- Secretaria de Vigilância em Saúde
3- Centers for Disease Control and Prevention

vanessaln@ioc.fiocruz.br[1]; mgumiel@ioc.fiocruz.br[1]; mmlima@ioc.fiocruz.br[1]; ebd6@cdc.gov[3]; cabello@ioc.fiocruz.br[1]; almeidace@ioc.fiocruz.br [1] ,jcosta@ioc.fiocruz.br[1]

Multidisciplinary research based on morphology, biology, ecology, isoenzymes, and mitochondrial DNA variation showed that the remarkable chromatic variations observed across the geographic distribution of *Triatoma brasiliensis* indeed constituted a species complex. Two members of the *T. brasiliensis* species complex (*T. melanica* and *T. juazeirensis*), were shown to be truly independent evolutionary units, and genetic evidence suggested another two had diverged sufficiently to be considered subspecies (*T. brasiliensis brasiliensis and T. b. macromelasoma*). Morphologic biologic and genetic features exhibited by *T. b. macromelasoma* placed it as an intermediate form between two members of this species complex: *T. brasiliensis* and *T. juazeirensis*. Collections were carried out along the supposed boundary where intermediate forms could be found, comprising thirteen municipalities and three different biogeographic regions of Pernambuco state so called: 1- Sertão do São Francisco, 2- Sertão do Araripe and 3- Agreste. We characterized these morphological forms and correlated them with the geographic distribution. A total of 192 specimens have been captured, and thirteen distinct phenotypes were detected based on the thorax and legs chromatic variations. Of these thirteen phenotypes, twelve could be found in the Sertão do Araripe Region, with the *T. b. macromelasoma* phenotype as the most common (24%). In the São Francisco Region, we found the second most diversified variation, where ten phenotypes could be found; the intermediate form between *T. b. macromelasoma* and *T. juazeirensis* was the most common (22 %) followed by *T. b. macromelasoma* (20%). These and other previously obtained results led us to

suggest that transgressive segregation might have been the mechanism by which some novel morphotypes arose in possible hybrid zones. The detailed and precise characterization of the phenotypic variations of the *T. brasiliensis* species complex is of great importance for monitoring the Chagas disease vectors in the studied areas.

VE25 - COLLECTION OF *Triatoma lenti*, *Triatoma pseudomaculata*, *Triatoma sordida* AND ISOLATION OF *Trypanosoma cruzi* STRAIN

ALVES, R.T.[1], MENDONÇA, V. J.[2], ARAÚJO, R. F.[3], ROSSETO, L. A.[1], RICCI, T. A.[1], FERNANDES, M. Z. T.[1], ROSA, J. A.[1]*

[1]Faculdade de Ciências Farmacêuticas/UNESP/Araraquara, SP, [*] Bolsista do CNPq
[2]Programa de Pós-Graduação em Parasitologia/UNICAMP/Campinas, SP
[3]Secretaria de Saúde do Estado da Bahia/SESAB/Salvador, BA

Triatoma lenti was described with *Triatoma pessoai* and *Triatoma bahiensis* in 1967 by Sherlock & Wilson in the State of Bahia. *T. lenti* and *T. pessoai* were found naturally infected by *Trypanosoma cruzi* and they have been easily maintained in the laboratory. However, only *T. lenti* species was considered valid for Lent & Wygodzinsky (1979). *Triatoma sherlocki* occurrence has also registered only for that state, the District of Santo Inácio. The objective of this work was to collect species of triatomines in Bahia, in order to maintain colonies in the laboratory and isolate strains of tripanosomatideos. The collection of triatomines was held in rural areas of the region of Boquira and Macaúbas, in intradomiciliary and peridomiciliary areas and assisted by the Health department. The feces of the insects collected were examined by abdominal compression. The collection was held at altitudes of 747, 755, 780 and 829 meters. We found 90 specimens of *T. lenti*, 324 of *T. sordida* and one of *T. pseudomaculata* in home. Four specimens of *T. lenti* and two of *T. sordida* showed the presence of Trypanosomatidae. One strain of *T. cruzi* was isolated in Swiss albino mice after intraperitoneal inoculation of feces obtained from a specimen of *T. lenti*, collected near the pigsty. From the *T. sordida* specimens collected at home, Rosa et al. 2004 had isolated 12 strains of *T. cruzi*, confirming the importance of this triatomine as a vector of *T. cruzi* in Bahia. This is the first record of *T. cruzi* isolation from *T. lenti*, since Sherlocki & Wilson (1967) did not characterize the Trypanosomatidae observed in the infected specimens. This work was supported by Fapesp (2006/02778-5), Health department of the State of Bahia and CNPq.

VE26 - The use of PCR multiplex assays to identify phlebotomine specimens naturally infected by *Leishmania (Leishmania) infantum chagasi* and *Leishmania (Viannia) braziliensis* in endemic Brazilian areas of visceral and cutaneous leishmaniasis

Daniela de Pita-Pereira[1], Thais Araújo Pereira[1], Cristiane Viana Mendes[1], Larissa Resende Vieira[1], Maria Angelica Cardoso[1], Getúlio Souza[2,3], Adriana Zwetsch[4] Carlos Roberto Alves[1], Elizabeth Ferreira Rangel[4], Reginaldo Peçanha Brazil[5] and Constança Britto[1],

[1] Laboratório de Biologia Molecular e Doenças Endêmicas, Instituto Oswaldo Cruz, FIOCRUZ, RJ, Brasil
[2] Seção de Reservatórios e Vetores, Laboratório Central de Pública do Rio Grande do Sul, Fundação Estadual de Produção e Pesquisa em Saúde, Secretaria Estadual de Saúde do Rio Grande do Sul, RS, Brasil
[3] Núcleo de Controle de Roedores e Vetores, Seção de Zoonoses, Coordenadoria Geral de Vigilância em Saúde, Secretaria Municipal de Saúde de Porto Alegre.
[4] Laboratório de Transmissores de Leishmanioses, Instituto Oswaldo Cruz, FIOCRUZ, RJ, Brasil
[5] Laboratório de Bioquímica e Fisiologia de Insetos, Instituto Oswaldo Cruz, FIOCRUZ, RJ, Brasil

We describe the application of PCR multiplex assay coupled to non-isotopic hybridization to evaluate natural infection rates in *Lutzomyia* spp. by *Leishmania* parasites in different Brazilian areas. The insect captures were performed in the following regions: Rio de Janeiro (CL areas); Corumbá (VL area); Porto Alegre(CL area). The species were separated in male and female and grouped into pools of 10 *Lutzomyia* individuals from the same specie and locality. The PCR multiplex was designed for the detection of both a genomic sand fly sequence specific for the genus *Lutzomyia* and the conserved region of *Leishmania* spp. kinetoplast minicircle DNA. PCR positive results for parasite detection were further analyzed

by hybridization with biotinylated specie-specific probes. Positive results for infection with *L. (V.) braziliensis* were achieved in 5 out of 32 female pools of *Lu. intermedia* and in 3 out of 5 *Lu. migonei* captured in Rio de Janeiro (Pita-Pereira *et al*, 2005). In Porto Alegre, infection by *L. (V.) braziliensis* was identified for the first time in *Lu. neivai* (3/27). In Corumbá, area with visceral leishmaniasis, we confirmed the participation of *Lu. cruzi* (2 positive pools out of 27) in the transmission of *L. infantum chagasi* and first described natural infection by this parasite in *Lu. forattinii* (1 out of 27 pools) (Pita-Pereira *et al*, 2008). In conclusion, if one considers at least one infected insect in each positive pool, it was possible to infer an infection rate of 2% (8 out of the 40 female groups formed) in the analyzed samples from Rio de Janeiro; 1.1% for sandflies collected in Porto Alegre; 1.5% and 0.7% for *Lu. cruzi* and *Lu. forattinii*, respectively, in Corumbá. The data achieved herein contribute to better understand the leishmaniasis ecoepidemiology in distinct endemic areas from Brazil.

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VE27 - DEVELOPMENT OF *LUTZOMYIA LONGIPALPIS* (DIPTERA: PSYCHODIDAE) IN SOIL FROM HENHOUSES

MARTINS,K.A [1], MENDES-SOUSA,A [1], ALEXANDER,B [2] ,BATES,P.A [2], MORAES,W [1], CAVALCANTE,R.R [1].

[1]-Universidade Federal do Piauí,Teresina, Brazil ; [2]- Liverpool School of Tropical Medicine,Liverpool,England

During the last 25 years visceral leishmaniasis caused by *Leishmania infantum* has become an increasing problem in many Brazilian cities. The vector *Lutzomyia longipalpis* s.l., is abundant in henhouses, chicken-rearing being a common practice in the marginal neighbourhoods where most VL occurs.As such, chicken-rearing has been cited as a risk factor for humans acquiring the disease. Although *Lu. longipalpis* bites chickens avidly and henhouses are foci of its courtship behaviour, there is no evidence that these shelters provide breeding sites for the insects, possibly because the high ammonia content of chicken manure precludes larval development. To determine whether *Lu. longipalpis* could breed in such an environment, we reared these insects in media consisting of different relative proportions of a standard laboratory colony diet, mixed with a stock sample of chicken manure whose chemistry (pH, % organic material and NH3 content) had been analysed. Fifteen plaster-lined pots containing a diet/manure mixture were set up for five relative proportions of the two components (0:100, 25:75. 50:50, 75:25 and 100:0).172 A gravid female sand fly was introduced into each pot and the number of eggs laid noted. The appearance of different larval and pupal stages was noted, together with adult emergence date, male:female ratio of progeny, number of adults produced per egg batch and total development time. There was no significant difference between any of the groups at $P \leq 0.05$ for any of the variables. Female sand flies were able to oviposit in a medium consisting entirely of chicken manure and immature stages developed at the same rate and in equal numbers in this environment as those reared in a standard laboratory diet. Henhouses could provide thus breeding sites for *Lu. longipalpis,* further evidence of their importance as factors in the establishment of urban VL foci in Brazil.

VE28 - LIFE CYCLE OF *PHYTOMONAS SERPENS* IN THE HEMIPTERAN INSECT HOST *ONCOPELTUS FASCIATUS*

Dias, F.A.[1], Alves e Silva, T.L.[1], Sathler, T.M.[1], Dutra, F.L.[1], Zimmermann, L.T.[1], Gomes, M.T.[1], Vieira, D.P.[1], Lery, L.M.[2], Bisch, P.M.[2], Attias, M.[2], Santos, A.L.[1], Souto-Padrón, T.C.[1], Lopes, A.H.[1]

[1]Instituto de Microbiologia Prof. Paulo de Góes, [2]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

Trypanosomatid-free *Oncopeltus fasciatus* insects were obtained by treating their eggs with sodium hypochlorite and ethanol; these insects were used throughout the present study. *Phytomonas serpens* promastigotes were injected into the thorax of adult *O. fasciatus*. At different periods of time after the injections, the hemolymph was collected by cutting off metathoracic legs and gently pressing the abdomen. Profuse amounts of parasites were observed in Giemsa-stained smears from the hemolymph. These insects were carefully dissected and their intact salivary glands extracted, fixed and examined, by means of scanning electron microscopy. Parasites were observed attached to the basal lamina, through the flagellum and the cellular body. On the other hand, the invasion of the basal lamina occurred only via

protozoan body. Parasites were also observed under the basal lamina, attached to the salivary outer surface of the gland, as well as entering the lumen of the glands. To identify a potential ligand for the parasites, salivary gland polypeptides were separated by 2D-PAGE, transferred to PVDF membranes and incubated with live biotinylated parasites. The development of the reaction showed that parasites bound to a 130 kDa polypeptide (p130). To identify the p130 polypeptide, the spot was cut from 2D gels and subjected to trypsin digestion. The peptide mass fingerprint spectrum was acquired and calibrated. Sixteen peptides perfectly matched peptides predicted for the human laminin 5 β-3 chain, representing 23% of the laminin sequence. Parasite entrance into the space between the basal lamina and the epithelium probably occurs through basal lamina disruption, as evidenced by lesions that remained at the gland surface. Interestingly, a 115 kDa surface protein of *O. fasciatus* salivary glands was cleaved after incubation with live *P. serpens* parasites, generating some low molecular polypeptides, as observed by SDS-PAGE. The addition of the cysteine peptidase inhibitor E-64 hindered this proteolytic cleavage.

Supported by: CNPq, FAPERJ, CNPq/PIBIC-UFRJ, CAPES.

VE29 - SALIVARY GLANDS OF *Rhodnius prolixus* (Hemiptera, Triatominae): AN ANATOMICAL STUDY OF ALL LIFE STAGES

ANHÊ, A.C.B; PIMENTA, P.F.P.

Laboratory of Medical Entomology – Instituto René Rachou / FIOCRUZ – M.G
Av. Augusto de Lima 1715 - CEP 30190-002 - Belo Horizonte, MG, Brazil.
e-mail: anhe@cpqrr.fiocruz.br

Triatomines are hematophagous insects, vectors of *Trypanosoma cruzi* and *Trypanosoma rangeli*. In all their life stages, during blood feeding process, they secrete saliva that abounds in substances that antagonize the hemostatic, inflammatory and immunological systems imposed by vertebrate hosts. In order to better understand the *Rhodnius prolixus* salivary gland development and functional structures, a study was carried out with nymphs (1st to 5th instars) and adults. The insects were from a well established colony from the Laboratory of Triatomines (IRR - FIOCRUZ). The organs were routinely dissected and processed for observations at Laser Scanning Microscopy (LSM) and Scanning Electron Microscopy (SEM). To observe the gland cytoskeleton by LSM, entire glands were incubated with FITC or rhodamine-phalloidins followed by the nuclear marker DAPI. Intact or opened glands were visualized by the SEM. The results showed that in general, the salivary glands have similar aspects during all the insect life stages, i. e., formed by one pair of lobe, each one composed by two lobules: the accessory and principal. The fluorescent phalloidin showed muscle layers formed by transversal fibers covering the entire organ, which was confirmed by SEM of the external surface. The nuclear marker unveiled secretory cells with two bulky nuclei. SEM of opened glands revealed internal aspects of the organ showing secretory cell surfaces. It was interesting to note that independently of the insect life stages, all glands have similar numbers of secretory cells. This fact suggests that the increase of the gland sizes observed in distinct stages occurs because of the growing of the cell volume (nucleus and cytoplasm). Mitotic cells were observed only in glands of adults and after their blood feeding. Further studies are being done in order to better understand the morphological characteristics of the salivary complex and to correlate them with the organ physiology.

Financial support: IRR/FIOCRUZ

VE30 - MORPHOMETRIC STUDY OF THE EVOLUTIONAL INSTARS AND MORPHOLOGIC STUDY OF EGGSHELLS OF *Tritatoma klugi* (HEMIPTERA: REDUVIIDAE) CARCAVALLO, JURBERG, LENT & GALVÃO 2001

ROCHA, C. S. [1], ROSA, J. A[1]

[1]Faculdade de Ciências Farmacêuticas – UNESP – Araraquara/SP

Triatoma klugi was described in 2001 by Carcavallo et al. and was found in the cracks of rocks around Nova Petropolis, State of Rio Grande do Sul, in Southern Brazil. Being a recent described species, this study aims to achieve the morphometry of 15 bugs of the five nymph instars and male e female adults, as well as the morphometric and morphological study of 50 eggshells by scanning electron microscopy (SEM). Analysis of a colony of *T. klugi* maintained in the Triatominae insectarium, located at the Faculdade de Ciências Farmacêuticas/ UNESP / Araraquara,

found that the eggshells have an opercular opening diameter of 0.661mm, length of 2.109mm and width of 1.290mm and by SEM we observed exocorials cells of regular sizes and variable shapes, with predominance of hexagons. The number of pores found per cell varied between 35 and 44, which differs from *T. rubrovaria*, a species that can be found in the same ecotope of *T. klugi*. In the description of *T. klugi* Carcavallo et al. (2001) measured the distance between the eyes, anteocular and postocular, and the diameter of the male and female adults eyes. In this study we evaluated the same parameters for all the evolutionary stages and also the head and abdominal length, as well as the three rostral segments, as proposed by Dujardin et al., 1999. Given that the description of *T. klugi* was not made by morphometric study of nymphs instars and the morphology of eggshells, this study aims to establish a better characterization of this species.

VE31 - CONFOCAL IMAGING OF LIVING *TRYPANOSOMA CRUZI* - VECTOR INTERACTION WITH CDTE QUANTUM DOTS STAINED.

C.S. Vieira[1], S.A.O. Gomes[1], A.A. de Thomaz[3], W.M. Faustino[3], Santos-Mallet J.R. [1],C.L. Cesar[3] and D. Feder[2].

[1]Laboratório de Transmissores de Leishmanioses, Setor de Morfologia, Ultraestrutura e Bioquímica de Artrópodes e Parasitos, IOC-FIOCRUZ-Rio de Janeiro; [2]Laboratório de Biologia de Insetos, GBG, Universidade Federal Fluminense-UFF; [3]Laboratório de Espectroscopia Resolvida no Tempo em Estruturas Quânticas,Instituto de Física "Gleb Wataghin", UNICAMP.

The real time imaging of living cells has been one of the biggest challenges for cell biology. Fluorescent markers have been a powerful tool to visualize cell processes but they can present some drawbacks. For example, organic dyes tend to be toxic to cells. It has been difficult to manipulate green fluorescent proteins (GFP) because they present a short fluorescent time. Semiconductor colloidal quantum dots have been, for the past two decades, incorporated in a wide range of applications from catalysis and optical sensors to biolabels. For this reason, simple, cheap and reproducible routes of synthesis have been the main goal of many research groups around the world. Quantum dots (QDs), have taken advantage in studies of cell biology, as an interaction parasite-vector, due to of the efficiency of the fluorescence and high photo stability of the semiconductor dots, as described in a novel class of biomarker**.** We reported here the use of fluorescent quantum dots for the study of *in vitro* and *in vivo* interaction of *Trypanosoma cruzi* and triatomines insects as *Rhodnius prolixus. T. cruzi* cells have not presented autofluorescence and the cell division had not been altered, being observed motile and intact parasites. Midgut cells of control insects presented a minor autofluorescence when compared with labeled midgut cells from insects QDs feed. *In vitro* experiments showed QDs labeled posterior midgut epithelial cells of *R. prolixus* with living *T. cruzi* also labeled attached to Perimicrovilar Membrane Matrix. This was the first time that QDs biomarkers had been used in a study of parasite-vector interaction.

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VE32 - STRUCTURAL ASPECTS OF THE SALIVARY GLANDS OF THE *Thyrsopelma guianense* (DIPTERA: SIMULIIDAE)

Chagas, A. C. & Pimenta, P.F.P.

[1]Laboratory of Medical Entomology –René Rachou Institute / FIOCRUZ – Av. Augusto de Lima 1715 – CEP 30190-002 - Belo Horizonte, MG, Brazil, e-mail: achagas@cpqrr.fiocruz.br

Studies of the salivary glands (SG) of North American backflies have contributed significantly for understanding the function of the saliva. The blackfly *Thyrsopelma guianense* is the main vector of onchocerciasis in Amazon Yanomami Amerindian areas. This study aimed to characterize the *T. guianense* SG by optical, laser confocal (LCM) and scanning electron (SEM) microscopies. The female SGs of newborn insects (after emergence from the pupae), 12 h, 24 h and 48 h were dissected and processed for the distinct techniques. Histological sections were routinely stained with PAS, mercury-bromophenol, osmium tetroxide and toluidin blue. For LCM, SGs were incubated with fluorescent lectins (RCA, WGA and LPL) followed by the nuclear marker DAPI. The blackfly SG is a single organ composed by a pair of identical lobes. The lobes are divided by proximal and distal regions. Nervous complexes or muscle fibers were not seen in the blackfly SG as in other insect SGs. Twelve hour old blackfly SGs

were more flaccid and empty when dissected and wrinkled when seen under the MEV. The content and SG epithelium changed according to the aging. The WGA lectin showed fluorescent dots over the entire organ. The secretory cell nuclei were evidenced with DAPI staining. The saliva products were observed only the in the distal and the end of the proximal regions of the two lobes. The SG histological sections revealed protein and lipid contents inside secretory cavities but not in the reservoir. In emergent blackflies, some PAS-positive material was seen in secretory cells and secretory cavities. However, in 48 h, large amount of PAS-positive material were seen and most of the nuclei were flattened against the cell walls. The DNA was detected in all parts of glands, but more intensely in secretory lobes than in the reservoir. This work is part of the investigation about the secretory compounds of the *T. guianense* SG. These studies are necessary for better understanding the role of SG components in the transmission *Onchocerca* parasites. Acknowledgements: The financial support of CNPq, FAPEAM, FIOCRUZ and FAPEMIG.

VE33 - DIFERENCIATION INFERRED BY SECOND INTERNAL INTERGENIC TRANSCRIBED (ITS2) OF *Anopheles triannulatus* s.l. SPECIMENS CAPTURED IN MATO GROSSO DO SUL

NEVES, A[1].; TUBAKI, R.M.[2].; TIRONI DE MENEZES, R.M.[2], MUCCI L.F.[2], MARRELLI, M.T[3] & MALAFRONTE, R.S[1,4].

Depto. de Doenças Infecciosas e Parasitárias, FMUSP; 2-Laboratório de Entomologia Médica, SUCEN, 3-Depto. de Epidemiologia, FSP/USP, 4-Laboratório de Protozoologia, IMTSP/USP.

Anopheles (Nyssorhynchus) triannulatus is widely distributed in Brazil and is considered a polymorphic species. Recently, it has been studied as a complex of four forms: *An. triannulatus s.s*., *An. halophylus* and two other forms. One of them is sympatric with *An. halophylus* and *An. triannulatus s.s*. in Central Brazil and the other form possibly occurs in Ecuador. There is no evidence that these differences characterize them as a cryptic complex or they are subspecies. ITS2 sequences deposited in Genbank (U92331, AF462377) showed different *Bsr*I enzymatic restriction patterns. In this study, ITS2 of captured species from Central Brazil region were analyzed. Larvae and adults of *An. triannulatus* s.s. were collected in S. Encarnação Farm, S Rita do Pardo municipality, Mato Grosso do Sul (MS), from March to May of 2005 and January to May of 2006. After morphological identification they were kept in isopropilic until DNA extraction. ITS2 amplification was made utilizing CP17 and CP16 primers. It was showed that ITS2 from MS specimens had a difference in their length (500 and 600 bp) corresponding to 21.3% (61/287) and 78% (224/287) respectively. *Bsr*I RFLP preliminary results revealed that both ITS2 sequences corresponding to Genebank were found in MS anophelines and 70.4% (74/105) corresponding to U92331 sequence. In addition, a polymorphism pattern in these ITS2 PCR products was noticed. These ITS2 will be sequenced to verify the existence of this polymorphism or they belong, in fact, to a complex of cryptic species. These results may be useful tool to help the classical taxonomic identification. Supported by LIMHC/49.

VE34 - *Rhodnius prolixus* Trehalase: Gene Prediction and Expression Analysis

Majerowicz, D., Alves-Bezerra, M., De Lima, V.V., Paula, I.F., Paiva-Silva, G.O., Gondim, K.C.

Instituto de Bioquímica Médica – UFRJ – Brazil

Trehalose, a glucose disaccharide, is the major carbohydrate of insect hemolymph. It is generally accepted that circulating trehalose must be cleaved to produce glucose that is taken up by cells. This reaction is catalyzed by trehalase and only scanty information is available about this step of sugar metabolism in insects. Using *Drosophila melanogaster* trehalase gene (CG9364) as query, *Rhodnius prolixus* genome trace archive database was searched with Blastn. The resulting traces were assembled using CAP3 and then a new Blastn was preformed, until the complete gene sequence was obtained. Blastx analysis confirmed high homology against insect trehalases already described (best hit: *Nasonia vitripennis* trehalase, identities=55%, positives=73%). *Rhodnius prolixus* trehalase gene (RpTreh) is predicted to be intronless and codifies a 630 aminoacid protein with expected molecular mass of 73 kDa and theoretical pI of 7.91. Protein sequence has two trehalase signatures (aminoacids 166-179 and 467-476). ClustalW alignment and Neighbor-joining phylogeny analysis showed great conservation among insect groups. *Rhodnius prolixus* trehalase is predicted to have a signal peptide, with cleavage site between aminoacids 19 and 20, and one

transmembrane domain at the C-terminal end. Protein sequence has also five putative N-glycosylation sites and 31 putative phosphorylation sites. RT-PCR analysis showed that RpTreh is expressed in all tissues investigated, both in fed and unfed females. Real-time PCR showed that gene expression did not change in ovary at days after blood meal or in oocytes of different lengths. These results indicated a housekeeping function for this gene product. This study intends to identify trehalase enconding genes and characterize their expression, in an attempt to clarify important steps of sugar metabolism during insect oogenesis. Suported by FAPERJ, CAPES, CNPq and CNPq-UFRJ/PIBIC.

VE35 - Differential splicing originates three chitinases in *Lutzomyia longipalpis*, containing or not the chitin binding domain

Ortigão-Farias JR; Di-Blasi T; Tempone AJ; Traub-Csekö YM

Laboratório de Biologia Molecular de Tripanossomatídeos e Flebotomíneos; Instituto Oswaldo Cruz; FIOCRUZ; Rio de Janeiro, RJ, Brazil. (joaorof@ioc.fiocruz.br)

A better knowledge of the *Leishmania*-sandfly interaction might lead to the development of new strategies to control leishmaniasis. We are searching for proteins putatively involved in the interaction of *Leishmania chagasi* with *Lutzomyia longipalpis,* the main vector of visceral leishmaniasis in South America. We found a gut-specific chitinase with high levels of transcription in female adults at 3 days after blood ingestion, indicating a possible role in peritrophic matrix (PM) degradation.

This chitinase is also expressed in larvae, where two alternative splicing forms were found. Both of them contain fragments from the last intron, creating earlier stop codons that interrupt the translation of the protein's chitin biding domain (CBD). These new enzymes might function in the digestion of chitin-rich food, similarly to the chitinase without CBD from *Tenebrio molitor* (Insect Biochem. Mol. Biol., 2006, 36, 789–800).

A cDNA fragment codifying for the chitinase under investigation was previously used for screening a genomic library, which identified a genomic clone containing the chitinase gene. The sequencing of this gene disclosed the presence of 4 introns from which only one seems to go through alternative splicing. There is a predicted promoter in the 5` flanking region of the gene which may be able to induce expression in adult female midgut when the PM thickness is being regulated. Interestingly, an orthologous gene from *Phlebotomus papatasi* contains a 5` UTR sequence identical to the beginning of the *L. longipalpis* chitinase RNA, which can be an indication of possible conserved regulatory sequences among different sandflies. Bioinformatics analyses also indicated the presence of an ecdysone responsive element in the sequenced 5` flanking region.

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VE36 - CHARACTERIZATION OF TRYPSINS IN *LUTZOMYIA LONGIPALPIS*, THE MAIN VECTOR OF VISCERAL LEISHMANIASIS IN BRAZIL

MACEDO-COUTO, R.[1], TELLERIA, E.L.[1], ARAÚJO, A.P.O.[1], D'AVILA-LEVY, C.M.[1], TRAUB-CSEKÖ, Y.M.[1]

[1]Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brazil.

We are studying *Lutzomyia longipalpis*, the main vector of visceral leishmaniasis in Brazil. In sandflies of the Old World midgut enzymatic activity during blood digestion is one of the obstacles that *Leishmania* must surpass to succeed in establishing infection. Our preliminary results of enzyme activity assays using dissected guts suggest that *L. longipalpis* females at 48 hours after infection with *Leishmania chagasi* have lower trypsin activity than non-infected insects. We have previously described two trypsin cDNAs of *L. longipalpis*: one (*Lltryp1*) has a bloodmeal induced transcription pattern while the other (*Lltryp2*) has a constitutive pattern. In order to study trypsin expression profile, we designed peptides specific for each of the two trypsin aminoacid sequences and these were used to produce polyclonal antibodies. We have used *Lltryp1*-peptide antibody in Western blot experiments using insect midguts or carcasses. Bands of approximately 28 kDa were detected in midgut samples between 2 and 24 hours after blood ingestion, but not in carcass samples. We are presently performing experiments with the *Lltryp2*-peptide antibody. In-gel protease activity of insect midgut preparations at different times after feeding was also studied. Gel incubation in buffer solutions with different pH

values showed pH 8.0 to be optimal for proteolysis. Gel polymerized with gelatin, hemoglobin, casein or BSA showed a diverse pattern of bands for each substract, as well as different bands produced by midguts and carcasses. Gel incubation with specific inhibitors indicates that the major proteolytic activity is due to trypsin-like enzymes. Our results suggest that at least one trypsin is induced by bloodmeal. Hence, it is our aim to find if *Leishmania-Lutzomyia* interaction can interfere in trypsin expression or activity.
Financial support: CNPq and FAPERJ.

VE37 - THE PHYSIOLOGY OF THE MIDGUT OF *LUTZOMYIA LONGIPALPIS* (LUTZ & NEIVA, 1912): pH IN DIFFERENT PHYSIOLOGICAL CONDITIONS AND MECHANISMS INVOLVED IN ITS CONTROL

SANTOS, V.C.[1], ARAUJO, R.N.[1], MACHADO, L.A.D.[1], PEREIRA, M.H.[1], GONTIJO, N.F.[1]

[1] Universidade Federal de Minas Gerais, Minas Gerais, Brazil.

Nutrient digestion and absorption after blood feeding are important events for *Lutzomyia longipalpis*, which use these nutrients to produce eggs. In this context, the pH inside the digestive tract is an important physiological feature since it can markedly influence the digestive process as well as interfere with *Leishmania* development in infected phlebotomines. It was described previously that unfed females have an acidic midgut (pH6). In this study, the pH inside the midgut of bloodfed females was measured. The abdominal midgut (AM) pH varied from 8.15±0.31 in the first 10h post-bloodmeal to 7.7±0.17 after 24h. While the AM was alkaline during blood digestion, the pH in the thoracic midgut (TM) remained acidic (5.5-6.0). In agreement with these findings, the enzyme α-glucosidase, which optimum pH is 5.8, is mainly encountered in the acidic TM. The capacity of unfed females to maintain the acidic intestinal pH was also evaluated. Our results showed the presence of an efficient mechanism that maintains the pH almost constant at about 6 in the midgut, but not in the crop. This mechanism is promptly interrupted in the AM by blood ingestion. RT-PCR results indicated the presence of carbonic anhydrase in the midgut cells, which apparently are required to keep the pH6 in the midgut of unfed females. Investigations on the phenomena of alkalization, observed after blood ingestion, indicated that two mechanisms are involved: in addition to the alkalization promoted by CO_2 volatilization there is a minor contribution of a second mechanism not yet characterized. Some inferences concerning *Leishmania* development and pH in the digestive tube are presented.

VE38 - THE ROLE OF SALIVARY NITROPHORINS IN THE INGESTION O BLOOD BY THE TRIATOMINE BUG *RHODNIUS PROLIXUS*

PAIM, R.M.M.[1], ARAUJO, R.N.[1], SOARES, A.C.[1], GONTIJO, N.F.[1], LEHANE, M.J.[2], PEREIRA, M.H.[1]

[1] Departamento de Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Caixa Postal 486, 31270-901, Belo Horizonte, MG.
[2]Liverpool School of Tropical Medicine, LSTM, Liverpool, UK.

Rhodnius prolixus is a vector of *Trypanosoma cruzi*, the causative agent of Chagas' disease in Americas. They feed blood in all nymphal stages and adults. In order to enhance the feeding efficiency, they produce several bioactive molecules in their salivary glands and inject them into the host skin during the feeding process. Among these molecules, the most abundant are the nitrophorins (NPs), a class of hemeproteins capable to bind nitric oxide. In this work, we reduced the expression of the four NPs in the salivary gland of *R. prolixus* by RNAi and evaluated the feeding behavior of knockdown and control nymphs on the dorsal surface and the lateral tail vein of the mice. Salivary mRNA from the four NPs was reduced by more than 99%. Saliva from knockdown nymphs also presented 82% less hemeproteins and three fold longer coagulation time. The amount of proteins was not reduced and SDS-PAGE analysis showed that bands with approximately 20 kDa (NPs estimated molecular weight) appeared weaker in the saliva from knockdown group. After feeding on the dorsal surface, nymphs from both groups had similar weigh gains. However, knockdown nymphs had lower ingestion rates needing longer total contact time to ingest the same amount of blood. The ingestion rate was influenced by parameters related to probing and frequency of the cibarial pump. No differences were observed between groups fed on the tail vein. When the feeding site was compared, nymphs fed on the tail vein had higher effective ingestion rates, lower number of

interruptions and higher frequency of the cibarial pump in comparison to bugs fed on the dorsal surface. The results indicate that salivary NPs are important for the alimentary efficiency in small diameter vessels and that the blood flow directly affects the triatomine feeding performance. Supported by FAPEMIG, CAPES and CNPq.

VE39 - FATTY ACID METABOLISM IN *RHODNIUS PROLIXUS* INFECTED WITH *TRYPANOSOMA CRUZI*

Bittencourt-Cunha, PR; Folly, E; Paiva-Silva, GO; Sorgine, MHF and Atella GC;

Instituto de Bioquímica Médica – IBqM – CCS - UFRJ

Trypanosoma cruzi is hemoflagellate that employs a wide variety of mammalian hosts and hematophogus insects in its life cycle. In humans, *T. cruzi* is found as both an intracellular form, the amastigote, and as a trypomastigote form in the blood. Under natural condition this parasite is transmitted to the vertebrate host by triatomine insects. In the vector the parasite reproduces asexually.

The *T. cruzi* development is confined to the insect gut. We have previously demonstrated that *Rhodnius* midgut is the main organ in fatty acid absorption. Now we are studying the presence and variation of expression of the fatty acid binding-protein (FABP) in *Rhodnius'* midgut infected with *T. cruzi*. Additionally, we investigate the possibility of the infection with *T. cruzi* affect other tissues, like ovary and fat body.

In order to investigate the expression of FABP, adult females were fed with blood of rabbits containing *T. cruzi*. In different days after feeding, five insects were dissected and RNA was extracted. A real time PCR was made and statistic analysis using ANOVA test.

Preliminary results suggest that FABP expression is 7 times higher in the midgut infected with *T. cruzi* in the first day after feeding. In the other organs, we also observed a variation in the expression; in the ovary, the expression was 4 times higher but in the fat body was 5 times lower in comparison with the non-infected tissues. The present results suggest that *T. cruzi* is able to modulates the lipid metabolism in different tissues of the insect even though it doesn't reach the hemolymph.

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VE40 - STRATEGY FOR IDENTIFICATION OF THE PROTHORACICOTROPIC HORMONE (PTTH) GENE IN *RHODNIUS PROLIXUS* (STAL, 1859)

OLIVEIRA, A.[1]; NOGUEIRA, F. B.[1]; ROMANHA, A. J.[1]; BORGES, E. C.[2]; MACHADO, E. M. M.[2]

[1] Centro de Pesquisas René Rachou, Belo Horizonte. Brazil
[2]Universidade Federal de Ouro Preto, Ouro Preto, Brazil

The diseases there are transmitted by blood sucking bugs constitute a major concern for world's health. The restrict haematophagy of triatomine bugs make them a potential target of studies, once they are vectors of Chagas disease. The understanding of physiological and biochemical mechanisms of prothoracicotropic hormone (PTTH) activity during the development of *Rhodnius prolixus* larvae can provide a new method for growth control in this bugs. The main objective of this work is isolate and characterize the triatomines PTTH gene, and also evaluate its differential expression in uninfected and *Trypanosoma cruzi* infected insects. For the gene isolation and characterization, twenty fifth instar larvae, of *R. prolixus* were decapitated four days after blood meal and had their brain excised for mRNA extraction and posterior cDNA conversion. In order to design degenerated primers, the software MEGA 4.0 was utilized for the alignment of amino acid sequences that encoded PTTH gene in eight different insects species: *Helicoverpa armigera* (AY286543); *Helicoverpa zea* (AY172670); *Heliothis virescens* (AY172671); *Manduca sexta* (AY007724); *Bombix mori* (D90082); *Samia cynthia ricini* (L25668); *Antheraea pernyi* (U62535); *Hylophora cecropia* (AF288695). PCR reactions, using combinations of the eigth degenerated primers created, were made and one fragment of the expected size (approximately 600bp) was amplified, posterior purified for insertion in a plasmid for cloning. After the cloning process, the fragment was purified from the positive bacteria, submitted to sequencing in an automatic sequencer MEGA BACE and analysed using Phrad e Phrep, being only those that had Phred ≥ 20 considered. The sequences were compared with NCBI database and showed high similarity with ribosomal protein s4e (Max. Identity 99%, e-value 0,0, total score 809), presents in the salivary gland of *R. prolixus.* New experiments, using different methodologies of RNA extraction, other times of decapitation and other

degenerated primers combinations, will be performed. (Finantial Support: CPQRR/FIOCRUZ-CNPq-FAPEMIG)

VE41 - OXIDATIVE STRESS IN THE MIDGUT OF THE VECTOR OF CHAGAS' DISEASE Rhodnius prolixus

Gandara, A.C.P.[1]; Oliveira, J.H.M.C.[1]; Oliveira, M.F.[1]; Fernandes D.C.[2]; Laurindo, F.R.M.[2]; Oliveira, P.L.[1]

[1]Instituto de Bioquímica Médica, Programa de Biologia Molecular e Biotecnologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 21941-590, Brazil.
[2] Vascular Biology Laboratory, Heart Institute (InCor), University of São Paulo School of Medicine, São Paulo, Brazil

The Chagas' disease vector *Rhodnius prolixus* ingests large amounts of blood in a single meal, being digested in the midgut and releasing heme, a toxic and pro-oxidant molecule. Reports have demonstrated that reactive oxygen and nitrogen species may be involved in insect´s immune response and eventually play a role on its vectorial capacity. Little is known about the involvement of blood digestion products on the insect´s redox physiology. ROS production was increased in starved insects, especially in the posterior midgut (PM) epithelium. Based on fluorescence microscopy and HPLC analysis of superoxide production, the fluorescence signal of the redox sensitive dye dihydroethidium was increased only in starved insects. A NADPH oxidase-like activity inhibited by superoxide dismutase (SOD) was measured and higher activities were found in PM compared with the anterior midgut (AM). Products of blood digestion like hemozoin, heme, iron and hemoglobin were quantified during all the blood digestion and their concentrations do not correlate with oxidative damage in tissues from starved insects, evaluated by TBARS assay. Taken together, the results indicate that blood meal affects ROS production in the midgut of *R. prolixus.* We propose here that ROS levels may be a compensatory antioxidant mechanism used by hematophagous insects to counteract the effects of a pro-oxidant diet. Supported by FAPERJ, CNPq, PRONEX, Howard Hughes Medical Institute and ICGEB.

VE42 - THE INFLUENCE OF THE INTESTINAL ENVIRONMENT IN THE FEEDING PERFORMANCE OF *TRIATOMA BRASILIENSIS* ON LABORATORY RAT AND SINANTROPIC HOST PUNARÈ *THRICHOMYS APEREOIDES*.

PEREIRA I. D. C. A. [1], ARAUJO R. N. [1], GUARNERI A. A. [2], SOARES A. C. [1], DIOTAIUTI L. [2], GONTIJO N. F. [1], PEREIRA M. H. [1].

[1] Departamento de Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Caixa Postal 486, 31270-901, Belo Horizonte, MG.
[2]Centro de Pesquisas René Rachou, Fiocruz, Caixa Postal 1743, 30190-002, Belo Horizonte, MG, Brasil.

An important point directly related to the vectorial capacity of *T. brasiliensis* is its competence to ingest blood from their hosts. Feeding competence is related to the blood ingestion rate that depends on features of the vector and the physiology of the host. We have recently shown that modulating the intestinal environment is decisive to determine blood meal size. In addition, preliminary experiments indicated a greater difficulty of triatomines to feed on *T. apereiodes*. *T. apereoides* is a wild rodent that lives in the xeric rocky scrubland of Brazil and is associated to triatomines from endemic areas of Chagas disease. Therefore, in the present work we performed a comparative study of the feeding performance between *T. apereoides* and *Rattus norvegicus*. Natural feeding analysis demonstrated that nymphs fed on punarè had lower ingestion rates and weigh gain. To avoid the influence of skin haemostatic reactions, we evaluated the feeding competence on artificial feeders with blood, erythrocytes suspension and plasma. The ingestion rate was lower for nymphs feeding diets containing *T. apereoides* erythrocytes while no difference was observed between the feeding parameters from nymphs fed with plasma from rat or *T. apereoides*. When the anterior midgut of nymphs were analysed immediately after the natural feeding, the main difference observed was the presence of several clusters of erythrocytes in the contents from nymphs fed on rats which were not seen in nymphs fed on *T. apereoides*. Assays performed *in vitro* confirmed that the *T. brasiliensis* anterior midgut contents are able to agglutinate rat erythrocytes, but have no activity over *T. apereoides* erythrocytes. The results indicate that the erythrocyte agglutination inside the anterior midgut may be one of the parameters that influence the feeding behaviour on

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