VE001 - **TRANSCRIPTOME ANALYSIS OF PARASITE INFECTED TSETSE FLY SALIVARY GLANDS REVEALS DIFFERENTIALLY EXPRESSED HOST AND TRYPANOSOME GENES**

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Tsetse flies (*Glossina* sp.) are the sole vector of human and animal trypanosomosis in sub-Saharan Africa. The trypanosome parasite is transmitted by an infected fly through salivary gland secretion at the bite site. Tsetse saliva contains compounds that enable fly blood feeding and facilitate parasite establishment in the vertebrate host. In the present study we investigated genes that are differentially expressed in *Glossina morsitans morsitans* salivary glands upon infection by *Trypanosoma brucei brucei*, and compared parasite genes expressed within the salivary gland environment to those in the mammalian host. We sequenced two RNA libraries from normal and *T. b. brucei* infected *G. m. morsitans* salivary glands. Quantitative PCR and Western blots were used to confirm the tsetse genes differentially expressed. In addition, the transcriptome contained parasite specific expressed genes was compared to a *T. b. brucei* blood stream stage specific RNA library. Our results show differential expression of tsetse molecules involved in cell division and remodeling suggesting there is intense tissue renewal occurring in the salivary glands upon infection. Moreover, there is a significant reduction in genes for secreted peptides, indicating that salivary composition is drastically altered. Parasite RNA-seq analysis indicates that there is a switch from amino acid metabolism in the salivary gland to glucose metabolism in the blood. Identification of tsetse proteins that are modified by trypanosome infections will shed light on the dynamics of host-parasite interactions in the salivary gland. *T. b. brucei* salivary gland stage-specific proteins will provide insights on metacyclic parasites molecules expressed to adapt to the salivary gland environment and prior to mammalian host interaction. **Supported by:**NIH-USA

VE002 - **INSIGHTS ON TOLL, IMD AND JAK/STAT IMMUNE PATHWAYS IN *LUTZOMYIA LONGIPALPIS*, THE MAIN VECTOR OF VISCERAL LEISHMANIASIS IN BRAZIL**

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Insects are exposed to a wide range of pathogenic microorganisms during their life cycle. In order to survive infection risks, insects developed several structural barriers and immune responses. The insect immune system is basically composed of signaling pathways that control a diversity of effector mechanisms, thus allowing control of most infections. As found in other organisms, there are three main signaling pathways related to immunity in insects: Toll, IMD and Jak/STAT. Our purpose was to characterize these immune pathways in *L. longipalpis,* the main vector of visceral leishmaniasis in Brazil. For this, we transfected *L. longipalpis* embryonic LL5 cells with dsRNA for the repressor genes cactus (Toll), caspar (IMD) and pias (Jak/STAT). Using this approach, it was possible to correlate the expression of AMPs to corresponding pathways. We found that cecropin and defensin 4 were upregulated after transfection by dsRNA for cactus and caspar, showing redundant regulation via the Toll and IMD pathways. We also silenced the cactus gene in adult insects by dsRNA. Preliminary results indicate that unlike what was observed in LL5 cells, attacin and cecropin genes were downregulated after silencing. Furthermore, we assessed the response of immunity genes upon *Leishmania infantum chagasi* infection in adult insect. Cactus was upregulated at 48 hours after infection, demonstrating the role of the Toll pathway in response to *Leishmania* infection. The AMPs attacin, cecropin and defensin 4 showed significant increase in expression at 72 hours after infection, when the parasite is interacting closely with the sand fly intestinal epithelium. These results indicate that these genes are regulated by the sand fly immune response upon the presence of *Leishmania*, which may have an impact on parasite survival in the insect gut during infection. **Supported by:**Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)

VE003 - **ANTIBODIES AGAINST *LUTZOMYIA INTERMEDIA* AND *LUTZOMYIA WHITMANI* SALIVA IN CHICKENS PLACED IN AN ENDEMIC AREA FOR AMERICAN CUTANEOUS LEISHMANIASIS**

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American Cutaneous Leishmaniasis (ACL) is caused by the bite of an infected sandfly. *Lutzomyia intermedia* and *Lutzomyia whitmani* are vectors of *Leishmania braziliensis* in Brazil. The presence of chickens in an endemic area for ACL is considered important for sand fly attraction and probably a major risk factor for *Leishmania* transmission. So far, the role of chickens in ACL epidemiology has not been defined. The aim of this study is to determine if chickens placed in an endemic area for ACL develop antibodies against *L. intermedia* and *L. whitmani* saliva and, if so, whether these animals can serve as sentinels. To this end, 50 chickens were distributed in five houses (10 per household), in an endemic area for ACL in Bahia, and were naturally exposed to sand fly bites during eight months. To evaluate vector density, light traps were distributed and monitored monthly in the houses. Chicken blood samples were collected every two months to evaluate the presence of sand fly saliva antibodies by ELISA and Western Blot. Sera from experimentally immunized chickens were used as a positive control. Chickens naturally exposed to sand fly bites had a significant increase in antibody production against *L. intermedia* saliva. After eight months, over 50% of exposed animals became seropositive. Regarding antibodies against *L. whitmani* saliva, the seroconversion was faster and more intense, reaching 50% of seroconversion after 4 months and 75% after eight months. There was no correlation between specific anti-saliva antibody for both species when compared with sand fly density. Both species strongly recognized a 45 kDa protein from the yellow family protein and another protein of 62 kDa. These results indicate that chickens naturally exposed to *L. intermedia* and *L. whitmani* are able to develop specific anti-saliva antibodies and, in the case of *L. whitmani*, chickens can be considered as candidates for sentinel animals to monitor transmission of *L. braziliensis* in endemic areas. **Supported by:**Fiocruz; iii (Instituto de Investigação em Imunologia)/INCT

VE004 - **TEMPERATURE-DEPENDENT OVIPOSITION AND EGG ECLOSION IN *RHODNIUS PROLIXUS***

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Chagas disease, caused by *Trypanosoma cruzi*, is one of the 13 most neglected tropical diseases worldwide, and according to the World Health Organization, the major cause of failure in the global fight against these diseases is the lack of knowledge about the biology of vectors and parasites, and one of the main control strategies is to combat the vectors. Considering that the rate of development of insect populations is directly dependent on characteristics related to the growth of immature stages, in which the main regulating factor is temperature, we decided to examine the egg-laying behavior of *Rhodnius prolixus* (insect vector of Chagas disease) at different temperatures. Females were kept in temperatures ranging from 10ºC to 40ºC, the number of eggs collected and rates of eclosion assessed. Our results showed that the egg-laying of *R. prolixus* females occurred in temperatures ranging from 18ºC and 38ºC and the highest numbers of eggs were laid between 22ºC and 34ºC. Egg hatching did not occur at temperatures under 24ºC and above 36ºC. We coupled our empirical data to mathematical modeling and designed temperature-dependent growth rate and carrying capacity equations for *R. prolixus* and integrated these results through a modified Verhulst equation to evaluate *R. prolixus* population dynamics regarding the temperature change. In order to have a spatial overview of the potential area of spread of *R. prolixus*, we gathered temperature data from the International Panel for Climate Change (IPCC) for both current and future weather conditions in the Americas and applied our model to spatial datasets and obtained suitability maps. **Supported by:**CAPES, FAPERJ, CNPq, INCT-EM

VE005 - **TSETSE SYMBIONT, *SODALIS GLOSSINIDIUS,* IN THREE *GLOSSINA* SPECIES COLLECTED IN MURCHISON FALLS, UGANDA: ACQUISITION AND DIVERSITY**

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Tsetse flies (*Glossina* spp.) transmit trypanosome parasites and in addition harbor a double-stranded DNA virus and two symbiotic bacteria. *Sodalis glossinidius* present a beneficial interaction with the host, but under natural conditions in field only a small percentage of flies have *Sodalis* infections. We are interested to understand how diverse *S. glossinidius* bacterium are in circulating strains and how it is acquired by the adult fly in the field. Three different fly species *Glossina fuscipes fuscipes*, *Glossina morsitans morsitans*, and *Glossina pallidipes* live in sympatry in Murchison Falls in Uganda. Flies were collected from the same traps in ecologically distinct zones. To identify *S. glossinidius* presence we extracted gDNA from guts and performed PCR using *Sodalis* specific primers. The infection rate in *G. pallidipes* was 59%, in *G. morsitans* 29% and in *G. fuscipes* 29%. Three *Sodalis* genes (hemolysin, ompC, FliC) and a pseudogene from a phage were amplified from positive samples and PCR products were cloned and sequenced for variations. No variations were found in the sequences. However, control samples of *Sodalis* infected flies from Kenya and Tanzania showed also no variations suggesting that these *loci* were not variable enough to address our question. We analyzed *Sodalis* using 4 pairs of specific primers designed on microsatellite repeat sequences in order to investigate the diversity among *Sodalis* strains. We also used qPCR to quantify *Sodalis* infection. Our results indicate that flies harboring high infection levels present no *Sodalis* diversity while flies containing low infection levels present higher symbiont diversity. Host bloodmeal was identified by PCR in order to investigate whether *Sodalis* strains are obtained from a common host on which the flies may be feeding. The study of *Sodalis* acquisition and diversity in the field will lead us to further understand the complex equilibrium between the fly and its symbionts. **Supported by:**NIH-USA

VE006 - **DYNAMICS OF PARASITE TRANSMISSION IN SEMIARID CHILE: THE WILD CYCLE OF CHAGAS DISEASE**

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Natural oscillations in host-parasite dynamics may result from annual variation in host, vector and pathogen abundance and diversity that leads to annual variation in the force of infection. Therefore, to quantify the variation in demographic parameters and their integration in a temporal framework allow better understanding of the ecological basis of disease transmission. The wild cycle of Chagas disease in Chile is composed by the parasite Trypanosoma cruzi, wild vectors from the Mepraia genus, and species of native and peridomestic mammals. In hyper-endemic areas of Chagas disease, T. cruzi-prevalence can reach 46% in the vector M. spinolai and 61% in native mammal species. Even though these studies show high infection levels in host and vector populations, evidence is fragmented in space and time, and entirely circumscribed to instantaneous recordings. In this study, we used molecular techniques to evaluate the proportion of infected specimens in several species of native mammals and wild vector populations collected in a hyper-endemic area of Chagas disease in semiarid Chile between 2009-2013. This information allowed assessing inter-annual variation in the strength of infection, and detailed description of the ecological basis of T. cruzi-infection in a context of climate variation, host and vector abundance, and disease risk. Our results show strong interannual fluctuation in the level of vector (9.4–53.0%) and host (9.9–64.7%) infection, detecting coupled variation between vector and host infection for most of the years. We suggest that slight interannual variation in rainfall accounts for the coupled fluctuation in vector and host infection. However, abundant precipitation during some years results in asymmetric effects on host and vector populations, increasing the number of some mammal species, which in turn produces dilution effect in the infection level of mammals. Under this scenario, disease transmission toward vector populations may be diminished. **Supported by:**FONDECYT 11090086, 1085154, 1100339

VE007 - **PHOSPHOLIPIDS MEDIATE HEME CRYSTALLIZATION IN MIDGUT PERIMICROVILLAR MEMBRANES OF THE BLOOD FEEDING INSECT *RHODNIUS PROLIXUS***

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Hemozoin (Hz) is a heme crystal produced by some blood-feeding organisms, as an efficient way to detoxify heme derived from hemoglobin digestion. In malaria parasite *Plasmodium* and in helmint *Schistosoma*, Hz formation is mediated by lipid droplets, whereas in the triatomine insect *Rhodnius prolixus*, a vector of Chagas Disease, this crystal is essentially produced by extracellular phospholipid membranes named "perimicrovillar membranes" (PMVM) in the insect gut. Increasing evidence point out the central role of lipids as catalysts of biological heme crystallization, especially the neutral lipids. Here, we investigated the role of commercial phospholipids and lipids isolated from Rhodnius PMVM on heme crystallization. Phosphatidylethanolamine (PE), phosphatidylcholine (PC) and phosphatidylserine (PS), as well as total lipid extracts from PMVMs obtained from blood and plasma-fed Rhodnius were all capable to promote beta-hematin formation. Interestingly, the crystal morphologies from PE-mediated reactions were quite similar to those produced biologically, regardless the insect diet. Moreover, the kinetics of beta-hematin formation differ significantly among the inducers, with PE mediating the fastest reaction observed. In conclusion, heme crystallization is promoted by phospholipids, with PE acting as a key catalyst for this process in *Rhodnius* PMVM. **Supported by:**CNPq

VE008 - **INFLUENCE OF THE PRESENCE OF A PARASITE (*LEPTOMONAS WALLACEI*) IN THE GUT OF *ONCOPELTUS FASCIATUS* IN THE REPRODUCTION AND OVIPOSITION OF THESE INSECTS**

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Leptomonas wallacei is a monoxenic trypanosomatid, which was first isolated from the gut of the phytophagous hemiptera Oncopeltus fasciatus. O. fasciatus is an insect of the order Hemiptera, family Lygaeidae, being found in all the extension of the Americas, and widely used as an experimental model for several research groups around the world. This study aims to show if the presence of the parasite (L. wallacei) in the gut of the insect O. fasciatus may influence the development, reproduction, and oviposition of the insect, also noting the possible presence of deformities in the reproductive organs of male and female insects. To date, 20 females recently molted from fifth instar nymphs into adults (10 infected and 10 uninfected) were separated into individual plastic pitchers and kept together with sexually active males, in order to observe how long would take them to become sexually mature, and to analyze the behavior of copulation and oviposition. It was observed that it took the uninfected females in average 2 to 3 days to become sexually active. These females copulated 3 to 4 times a day in average. On the other hand, it took the infected females about 5 to 6 days in average to become sexually active and they copulated about 4 to 5 times a day. We also dissected 20 females (10 infected and 10 uninfected) and 20 males (10 infected and 10 uninfected), with the aim of evaluating possible deformities in the reproductive organs. We observed no deformities in the ovipositor of the females, but deformities were observed in the males of infected insects. In these insects, on some occasions, the testes had not their usual form, which looks like a wine glass; either it appeared appendices attached to these "glasses" or, in other cases, a type of most dramatic changes happened in these forms, where “buds” were observed instead of the “glasses”. Ongoing experiments are to provide better statistical analyzes. **Supported by:**FAPERJ, CNPq, CAPES, INCT-EM