# Epidemiologia - Epidemiology

## EP01 - Prevalence of *Trypanosoma cruzi*, *Leishmania braziliensis* and *Leishmania chagasi* infections in Brazilians Amerindians Tribes

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Chagas disease is the most prevalent endemic disease in the Occidental World, showing 18 millions people infected by *T. cruzi* in Latin America countries, from Mexico to Argentina (WHO, 1991). Visceral Leishmaniasis is caused by *Leishmania (Leishmania) braziliensis* transmitted by *Lutzomyia longipalpis*. In the last decades it has been observed emergency and re-emergency of this endemic disease in several regions in Brazil. The American Tegumental Leishmaniasis (LTA) is the sixth most prevalent endemic disease in the world (FUNASA, 1998). This is a zoonose affecting mainly wild animals and, in several occasions, man and domesticated animals. The Amerindians have been in contact with cinetoplastid protozoa for over 50 thousands years, whereas the European and African settlers have been in contact with these infectious agents for 500 years. In this study we determined the seroprevalence for the three cinetoplastid infections among four Amerindian tribes in the States of Minas Gerais and Espírito Santo, Brazil. The ELISA, hemagglutination and immunofluorescence tests were used. In addition we used the *Western blot* assay to determine the diagnosis in cases with conflicting results. We established standard profiles of bands for each protozoan infection: *T. cruzi*, 153, 68, 63 and 13 kDa; *L. braziliensis*103, 60 7 and 4 kDa; and *L. chagasi* 37, 31, 30 and 23 kDa. Altogether with the *Western blot*analysis, we revealed the real prevalence for each infection in 451 Amerindians in the four tribes: 0,9 % was positive for *T. cruzi* infections; 3,3 % for *L. braziliensis*, 5,1 % for *L. chagasi* infections. In addition, we observed mixed infections of *T. cruzi* and *L. braziliensis* in 0,9 % of the cases, plus 1,1% showing both Leishmania sp infections. Actually, 3,1% of the analyzed Amerindians had triple infections.

## EP02 - Detection of the natural infection by *Trypanosoma* spp.(Kinetoplastida:Trypanosomatidae)in rodents and marsupials(Mammalia:Rodentia and Marsupialia) of Madeira River,Rondonia State,Brazil.

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In Northern Brazil, the Rio Madeira is considered as one of the four main rivers to delimit endemic areas of Amazonian fauna (Wallace, 1849). Small mammals (specifically, rodents and marsupials) from the Madeira Valley are considered reservoir hosts of many endoparasites. Isolation of protozoan flagellates was made from small mammals captured on both banks of the upper Rio Madeira, between Cachoeira de Santo Antônio and Abunã, Rondonia. We examined 57 small mammals, including rodents: *Mesomys hispidus* two individuals (3,5%), *Proechimys* sp. 18 individuals (32%), and marsupials: *Micoureus demerarae* 28 individuals (49%), *Didelphis marsupialis* eight (14%), and *Marmosops* sp. only one individual (1,75%). Flagellate isolation was made from different tissues (skin, spleen, liver, blood, bone marrow and others) by cultivation in NNN medium and laboratory animal inoculation. Natural infection by *Trypanosoma* was detected in 14 individuals (25%), eight (57%) in *Micoureus demerarae* and six (43%) in *Didelphis marsupialis*. Flagellates morphologically compatible with *Trypanosoma cruzi* were isolated from culture of blood, buffy coat cells, bone marrow, spleen and liver of six (6/8, 75%) *D. marsupialis* and eight (8/28, 28,6%) *Micoureus demerarae*. Mixed infection with *T. cruzi* and *T. rangeli* was observed in two (7%) *Micoureus demerarae* and one (12,5%) *D. marsupialis*. It is known that some species of didelphid marsupials, such as *Didelphis marsupialis*, maintain the parasitism by *T. cruzi* without apparent disease or any important tissue lesion; this species also plays an important role in the cycle of Chagas Disease. Our data reports the circulation of *T. cruzi* and *T. rangeli* in didelphids of the Madeira region, and the high rate of natural infection by *T. cruzi* in these opossums. IBAMA permit No. 02024000293/04-14; Supported by INPA/FURNAS.

## EP03 - Chagas Disease seroepidemiological study at San Pedro, rural zone with high endemicity antecedents of Sucre state, Venezuela.

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In order to extend our knowledge about current situation of Chagas disease at Sucre state, Venezuela, we carried out an seroepidemiological study by passive and active search. In this sense, we revised Venezuelan Chagas Disease Control Program report (CDCP, 2000), and 1982 dead files of Sucre and Nueva Esparta Rural Endemies Direction. In the period 2001 august-december, 194 persons of both sex and different ages were randomly studied at San Pedro village, Santa Fé parish of Sucre municipality, $10^o$13'00"NL and $64^o$ 25'02"WL, still conserving appropriate ecoepidemiological conditions to disease establishment. Using ELISA technique

standardized in our lab, total antibodies anti-*Trypanosoma cruzi* were determined and Immunoblot for antibodies IgG anti-*T. cruzi* detection were used as confirmation criterion of doubtful sera. Santa Fé parish in 1982 showed total elimination of *Rhodnius prolixus* infestation and *T. cruzi* infection after CDCP effective campaign began in 1968, when their initial values were the highest of Sucre state (74% peridomestic and 28% domestic infestation indexes associated to parasitological indexes 18% and 8% respectively). By active search we found 26% seroprevalence, which triplicate official national records (8.1%, CDCP 2000) concerning the thirteen highest endemicity of Chagas disease states, not included Sucre state. 24% of individual lower than 20 years old resulted seropositives suggesting an active transmission. 90% of seropositives individuals lived in mud houses and 30% possessed domestic animals related to disease. These findings situate San Pedro locality of Santa Fé parish as current highest seroprevalence zone reported in Venezuela. Additionally constitute an epidemiological alarm of Chagas disease to take into account by competent sanitary institutions for reimplementation of corresponding control, prevention and epidemiological surveillance measures in the region.

Key words: Chagas disease, Seroepidemiology, ELISA .

## EP04 - Hematological and serum biochemical evaluation of dogs *Canis familiaris*(Linnaeus, 1758) naturally infected with *Hepatozoon canis* (James, 1905) in rural areas in the state of Rio de Janeiro-Brasil

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This investigation was accomplished at the Experimental Station W.O. Neitz, Parasitology Department, Veterinary Institute of the Federal Rural University of Rio de Janeiro and at the Laboratory of Veterinary Pathologic Clinic of the Federal Fluminense University, Rio de Janeiro. Blood samples from 24 dogs coming from rural areas of Barra de Pirai county and naturally infested with *H.canis* were collected every two weeks from June to December 2001. Hematological and biochemical examinations were carried out with blood and serum samples. The hemoparasite was detected by examinations of Giemsa-stained blood smears. In parasitologic assay, the hemoparasitism was evaluated by the presence of into-neutrophilics gametocytes typical of the specie. The hematology analysis was performed using the following parameters: globular volume, mean globular volume, total red blood cells and total white cells. Determinations of serum urea, creatinine, glucose, aspartate amino transferase (AST), alanina amino transferase (ALT), total proteins, albumin, globulins, calcium, phosphorus, total billirubine, fosfatase-alkaline and creatine kinase (CK) were carried out in a spectrophotometer using commercial kits. Indirect bilirrubine, globulines and the relation albumin/globuline were mathematically calculated. There were no statistical significant differences among the various parameters from blood samples of parasitised and nonparasitised dogs indicating that *H. canis* is an hemoparasite with very low pathogenicity in this area. This is an important observation in clinical veterinary since the other species of this parasite have been related as cause high mortality in dogs. Supported by CNPq/FAPERJ.

## EP05 - PREVALENCE OF *Trypanosoma cruzi* IN *Triatoma infestans* COLLECTED IN 16 COMMUNITIES OF COCHABAMBA, BOLIVIA

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Bolivia nowadays has the highest rates of Chagas disease infection in Latin America. About 1.1-1.8 million people are estimated to be infected by *Trypanosoma cruzi*, this representing over 15% of the country's population. In 2003, the national health authority of Cochabamba, Bolivia, launched a program aiming at the elimination of *Triatoma infestans*, the primary vector of Chagas disease. Since previous serological studies carried out in 16 communities of Cochabamba revealed 67.3% (204) positive individuals among the 303 tested for *T. cruzi* infection by indirect hemagglutination and indirect immunofluorescence assays, a pre-investigation was carried out prior to triatomine elimination efforts, consisting in the search for the presence of *T. infestans* in all human domiciles of these communities. Among the 44 houses and human dwellings, a total of 225 adult triatomines and nymphs were manually captured by standard methods (1 man/1 house/1 hour), revealing a triatomine infestation of human houses and dwellings between 17 and 47%. Feces of all captured triatomines were observed by microscopy for the presence of flagellates, spotted on sterile filter papers and stored at 4oC. All 221 positive samples (98.2%) were submitted to DNA extraction by maceration and boiling the feces-bearing filter papers in Tris-EDTA buffer. PCR reactions were carried out using primers S35 and S36 directed to the kDNA minicircles. All PCR-analyzed samples showed positive results as revealed by the presence of a 300 bp product upon agarose gel electrophoresis. PCR showed a 100% concordance with the microscopic analysis; no evidence of the presence of *T. rangeli* was revealed by PCR. The obtained PCR products are now being used as template for LSSP-PCR analysis in order to evaluate the existence of genetic variability of the kDNA mini-circles among the *T. cruzi* samples circulating in the distinct communities in Cochabamba.

## EP06 - PREVALENCE OF MARKERS OF *Leishmania chagasi* VISCERAL INFECTION AND DISEASE EXPRESSION IN HUMANS AND DOGS IN A RURAL ENDEMIC AREA (MUNICIPALITY OF PANCAS, ESPIRITO SANTO, BRAZIL)

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Here we study the extent of cryptic *L. chagasi* visceral infection in both human and dogs in order to provide a basis for future interventions which could assist in the control of the disease. Sera sampled from 189 humans and 112 dogs living in four rural locations (Sao Luiz I, Palmital, Roque, and Uba creeks) in an endemic area (Pancas, ES) were analyzed by ELISA and IFA as screening tests. The overall prevalence of Leishmania antibodies (ELISA) in the human population was 40% (76/189). Of the 177 individuals skin tested with leishmanin, 92 (52%) had indurations major/equal than 5 mm after 48-72 h. There was evidence of long subclinical latency with no smoldering disease in the seropositive cases. The overall prevalence of specific antibodies (ELISA) in the dog population was 62% (69/112), but the seroprevalences were highly variable in the different localities, ranging from 45 percent(Palmital) to 85% (Uba). The high sensitivity of ELISA contrasts with the low sensitivity (14,6 %;15/103) obtained by IFA (in this case, examining dog blood collected on filter paper). Problems in determining the specificity of serology will be discussed. Seropositive dogs also developed signs of early VL. These data confirm the continuing occurrence of transmission in humans in the state, indicating that the control programs probably fail because of (1) high incidence of infected dogs and (2) time delays between diagnosis and culling. Prevention of disease in dogs by immunization would be the best approach to control the transmission cycle of zoonotic VL. Supported by grants from FIOCRUZ, PRONEX-CNPq and FAPERJ (BRAZIL).

## EP07 - Quantitative *Toxoplasma gondii* oocyst detection by a modified Kato Katz test, using Kynioun staining (KKK), as compared to qualitative sugar flotation techniques in ME49 strain experimentally infected cats

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Toxoplasmosis is a disease caused by *Toxoplasma gondii*, an obligated intracellular parasite worldwide spread, capable of infecting all warm blooded animals. Felidae are the definitive hosts, with entero epithelial multiplication with subsequent sexual stages and oocyst production. All cats are susceptible, regardless of age, sex and bred, shedding oocysts to the environment through feces. We compared two techniques for oocysts detection in feces of experimentally infected cats, using a qualitative sugar flotation and a Kato Katz approach using subsequent Kynioun staining. Animals serologically negative to *T.gondii* received by gavage $5x10^2$ mice brain cysts of ME49 strain of *T.gondii.* Feces were daily collected from the 3th to the 30th day post-inoculation (p.i.). Oocysts were detected by two methods, the classic qualitative sugar flotation, as standard, and the modified Kato Katz stained by Kynioun (KKK). Briefly, 40 ml of nylon filtered stools were placed on microscope slide in a Kato well, with a relatively thin smear obtained by sliding over another slide. Both slides were dried and stained by classical Kynioun method and observed in optical microscopy. *T.gondii* oocysts appeared red, with preservation of internal details. In the experimentally infected cats, oocysts were detected from 7th to 15th day through flotation technique and KKK showed oocysts from 6th to 16th day, being more sensitive for a larger period, with permanent documentation. KKK also allowed semi quantitative estimation of oocysts per grama of feces, showing that the peak of excretion occurred in the 8th to 11th days after challenge. KKK also showed the advantage of a fewer feces manipulation, decreasing the possibility of environment and operator contamination. Those advantages suggest that this modified technique could be introduced in the search of oocysts excretion in feces of suspected animals.
This work was supported by LIMHCFMUSP49, CAPES and CNPQ.

## EP08 - Incidence of giardiasis in the Centro de Saúde Escola Germano Sinval Faria (Fundação Oswaldo Cruz - Rio de Janeiro) for one year

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Giardiasis, an intestinal protozoan infection caused by *Giardia intestinalis* is a most common human protozoal infection. This fact can be understood, because the faecal-oral transmission incidence of giardiasis is related to bad sanitary conditions. In the tropics, this spread must be quite direct because *Giardia* cysts are not extraordinarily resistant either to heat or drying. Althoughmore common where hygiene leaves most to be desired, *Giardia* infections remain a problem in industrialized countries, where the prevalence rate is 2-5%. The disease is mainly characterized by gastrointestinal symptoms. In the present study, the incidence of the giardiasis was determinated for 1 year from August 1,

2003 to August 6, 2004 in the Clinical analysis laboratory of the Centro de Saúde Escola Germano Sinval Faria of the Fundação Oswaldo Cruz through a retrospective study of the results of 1860 parasitological examinations. This survey has shown the high prevalence of giardiasis, found in 7,6% (142 cases) of the population studied. Individuals under 5 years old was found to be the most affected age group, furthermore, the incidence of giardiasis reduced with the increase of age. Although *Giardia* infection can occur in hosts of all ages, the incidence of infection is likely to be highest in the young. This is age group least likely to be concerned about sanitation and more susceptible to the infection. Animal studies have showed that immune response to *Giardia* infection can make the host less susceptible to reinfection corroborating to these findings.

## EP09 - A digital image processing system for the species identification of *Eimeria* spp. oocysts

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Seven distinct *Eimeria* species infect the domestic fowl, causing enteric diseases generically known as avian coccidiosis. Because different species can vary in pathogenicity, the precise discrimination is important for epizootiological studies. Oocysts of distinct species present differences of size, shape, thickness and color of the oocyst wall, etc. However, the correct dicrimination by visual inspection is severely restricted by the slight differences and overlap of characteristics among the different species. This work aimed at developing a process for oocyst identification and classification through computational microscopic image analysis. For this purpose, digital oocyst images, obtained from pure strains of each *Eimeria* species, were captured with a 4-megapixel CCD camera and used in an automated feature extraction and classification system. In order to identify the distinct oocyst species, we used shape and textural features (diameters, perimeter, area, curvature, texture and simetry), constituting a set of 13 features (Costa, L.F.& Cesar, R.M. Shape Analysis and Classification, Theory and Practice, CRC Press, 2001). Species classification was performed with a Bayesian statistical multivariate analysis, which considers the gaussian distribution function. From a total of 1,205 oocyst images, using 30% as a training set for the generation of the classification model, and 70% as a test set, the overall rate of correct species assignment was 88.69%. *E. necatrix* was the most difficult species to discriminate due its morphologic overlaps with *E. mitis* and *E. praecox*. Kucera and Reznicky (Folia Parasitol. Praha, 38:107-113, 1991), using size differentiation, also observed a high similarity among these species. In our study we extended the morphological analysis including shape and texture features, obtaining a final specificity of 69.79% for *E. necatrix*. We are currently developing a web-based interface that will allow for species diagnosis through the internet, with no biological samples being required.

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## EP10 - ECO-EPIDEMIOLOGICAL ASPECTS OF VISCERAL LEISHMANIASIS IN THE ATLANTIC RAIN FOREST REGION OF PERNAMBUCO, BRAZIL: PREVALENCE OF INFECTION AND SANDFLY FAUNA INVOLVED.

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Cutaneous and visceral leishmaniasis are endemic in all regions of Pernambuco State, northeast Brazil. The predominance of cases ocurring in "Sertão"region to visceral leishmaniasis (VL) and in "Zona da Mata", corresponding to Atlantic rain forest, related to incidence of cutaneous leishmaniasis. Human cases of VL were registered in São Vicente Ferrer municipality, located in the transition area of "Zona da Mata"with the "Agreste"region. An epidemiological study were carried out in two localities where ocurring the recent cases with the aims of characterize the pattern transmission and to identify reservoirs host and sandflies vectors involved in the zoonotic and silvatic cycles of transmission. The prevalence of infection was detected by delayed hipersensivity test (or Montenegro skin test) (DHT) and indirect immunofluorescence test (IFI) to anti-*Leishmania* antibodies. In the Mundo Novo locality was identified 39.2% (71/181) and 9.4% (17/181) of positivity, respectively. In the Mirim locality, 17% (46/270) to DHT and 8.8% to IFI. In the prevalence survey to dog infection by IFI, 33% of positivity was detected, although assintomatic in the majority and some few oligosintomatic. *Lutzomyia migonei* presents strong evidences as the vector involved and zoonotic transmission by the predominance in houses and peridomestic sites and also cinophily atribute. *L. longipalpis* was not found in the area. *L. complexa* is the species that predominates in silvatic ecotopes corresponding to remnant rain forest patches. More studies will be developed to complete the pattern transmission characterization and confirm some hypothesis.
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## EP11 - ISOLATION OF TRYPANOSOMA CRUZI STRAINS FROM TRIATOMA SORDIDA COLLECTED IN PERIDOMESTIC ENVIRONMENT IN SANTO INÁCIO, BAHIA

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The decrease of Chagas disease incidence in Brazil does not mean that the epidemiological vigilance is no longer necessary. The potential triatominae vectors need to be evaluated constantly (Dias, 2000). Corroborating to this statement, in the present study it is related the isolation of *Trypanosoma cruzi* strains from *Triatoma sordida* collected in July/2003. Nymphs and adults were captured inside a room besides a house located in the district of Santo Inácio, Bahia. Out of 18 *T. sordida* examined, 12 have had Trypanosomatidae forms in their intestinal contents which were inoculated in mice Mus musculus Swiss. Later on trypomastigotes forms were found in the animals blood which after being stained were identified as *T. cruzi*. Some morphological, biological and molecular parameters of the strains have been evaluated in mice and LIT culture medium. Precipitin tests were carried out with all the 12 positive *T. sordida* intestinal contents. The results have shown that eight individuals had their blood feed on cat, one on cat and dog and one on human. The ingested blood of two of them were not identified. According to the observed data, some procedures are necessary in Santo Inacio, such as exams on the local population in order to diagnose human Chagas disease, the search of triatomine in the domestic and peridomestic areas and a constant vigilance to monitor domestic colonisation of Triatomine species.
This research was supported by PADC/FCF

## EP12 - Isolation of *Leishmania (Viannia) braziliensis* from a human patient in the municipality of Rincão, São Paulo state.

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Four American Cutaneous Leishmaniasis (ACL) human cases were notified from 1998 to 2003 and two other cases in the first semester of 2004 in the municipality of Rincão. It is located in the northeast centre of São Paulo state. In order to identify the *Leishmania* species circulating on that region, one patient was submitted to protocols of isolation techniques. Punch biopsy and aspirated tissue were taken out from the lesion. The aspirated was inserted straight into a tube with saline plus antibiotics and centrifuged after three hours (1000g for 1 minute). Fragments and the pellet of the aspirated were inoculated individually into a biphasic culture medium (NNN-agar blood with LIT medium) and injected subcutaneously in one hind footpad of mice (BALB/c) and hamsters. Promastigotes forms were observed after eight days only in the tubes which the aspirated was inoculated. The isolate of Leishmania was analyzed by enzymes electrophoresis. The results have shown that *Leishmania braziliensis* is the species evaluated. Entomological surveys were performed about 50 meters from the edge of Mogi-Guaçu river, area where one confirmed ACL patient lives. Shannon trap and CDC light traps were used for two nights from 18:00h to 22:00h. The only sandfly species captured was *Lutzomyia neivai* (Pinto, 1926) (149 females and 79 males).

## EP13 - Surveillance of non-symptomatic malaria infections in a riverine area in Western Amazonia following mass-treatment

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The present study intends to ransom current aspects on the malaria epidemiology in a population studied for two years by Alves et al. (2002) at the Machado riverside (RO), searching to emphasize differences that might have occurred along time and to characterize the profile of this population after treatment of asymptomatic carriers of the parasite. A deeper knowledge on the asymptomatic malaria epidemiology is vital for the more effective control of the endemics. The low Machado riverside population is mostly native or people who have lived for many years in that region, with not much mobility and who develop subsistence economic activities. In the year of 2000, they were 858 individuals scattered on approximately 250 Km from the Machado river. Two collects (transversal cuts) were performed in intervals of 6 months between collects and the blood was analyzed through PCR for the presence of *Plasmodium vivax* and/or *P.falciparum*. 732 samples were obtained in the first collect (August/2003) and 593 samples in the second (February/2004). In this population, 56.7% of the inhabitants are not older than 10 years of age and 53.9% not older than 20. The sexual ratio (male: female) is approximately 1:1. The adhesion rate observed (313 individuals) was of 42.76% and the discontinuance of

some patients and the entering of some new ones (49.59%) may be mainly explained by the characteristic mobility of the individuals from this region. The presence of *Plasmodium* sp in 28.3% and a ratio between *P. falciparum*: *P. vivax* of 0.99 in the population was verified in the first cut. These proportions were reduced to 18.7% and 0.22, respectively in the second cut. From the samples studied, 7.1% and 1.9%, respectively for the first and second cuts presented positive amplification both for *P. falciparum* and for *P. vivax* (mixed infection). Our results are in agreement with results observed by Alves (2002) when we observed that in the dry season the infections by *P.vivax* were predominant while in the high-transmission period, the infections frequency by *P.vivax* and *P.falciparum* were similar. Other collects are planned for the next two years with the objective of better understanding the transmission dynamics of the parasite in the different climate phases of the region (rainy and dry). The cases distribution according to the geographic localization has also been evaluated.

## EP14 - Spatial analysis of recorded cases of human visceral leishmaniasis in Campo Grande, Mato Grosso do Sul, Brazil, 1999-2003

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Leishmaniasis has become a serious Public Health issue in several regions of the world, as the number of its new cases and foci has increased over the past decades. The first autochthonous case of human visceral leishmaniasis in South America occurred in an individual living in Porto Esperança, in the Brazilian municipality of Corumbá, classified as endemic by the Brazilian Ministry of Health. The spread of the disease since 1996, with human cases occurring in diverse regions of the state of Mato Grosso do Sul, and culminating with an high mortality-rate epidemic in the municipalities of Campo Grande and Três Lagoas, led to the need for identifying the dissemination and the possible routes of expansion of American visceral leishmaniasis (AVL) in Mato Grosso do Sul (Corumbá, Campo Grande, Três Lagoas), extending toward the state's border with São Paulo. In the present study, data on AVL from the Health Department of the Municipality of Campo Grande (SESAU) were gathered through the Brazilian Information System of Notifiable Health Events (SINAN). Confirmed cases of AVL from 1999 to 2003 in Campo Grande were spatially distributed by infectious source. Spatial analysis and Geographic Information Systems (GIS) are instruments that allow variables such as population and quality of life index of a given region to be spatially visualized through maps. In addition to this visual perception of the spatial distribution of a problem, the procedures enable epidemiologists to collect data on the occurrence of diseases. The distribution of cases of a disease configures a spatial pattern that allows information to be spatially analyzed. The relationship between the disease and the participation of environmental processes that may influence its dissemination or its source of infection can thus be measured. By analyzing point patterns and surface, area and GIS data, it is possible to infer how a given disease is spreading and what control measures should be proposedas with AVL in the present study. The concentrations of cases thus obtained have been found to differ from those reported in the literature.

# Imunologia - Immunology

## IM01 - Effect of Human apoptotic neutrophils phagocytosis by *Leishmania amazonensis* infected macrophage

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Neutrophils are programmed to undergo apoptosis and provide the first line of defense against infections. Macrophages uptake apoptotics cells releasing antiinflammatory mediators. In the present study, we evaluated the effect of human apoptotic neutrophils phagocytosis by human *Leishmania amazonensis* infected macrophage. The preliminary results showed an increase on the frequency of macrophage infection by *Leishmania* as well as their parasite burden in the presence of apoptotic neutrophils. We observed that this increase is dependent on TGF$\beta$ and $PGE_2$ released by macrophages, since the presence of anti TGF$\beta$ and or Indometacine abolished the effect observed. On the other hand, when necrotic neutrophils were used, there was a reduction on the frequency of macrophage infection as well as parasite burden. This reduction was dependent on TNF$\alpha$ and Neutrophilic Elastase (NE) release, since the presence of anti TNF$\alpha$ and or anti elastase abolished the effect observed. The control with Jurkat cell showed an increase on the frequency of macrophage infection by *Leishmania* as well as their parasite burden in the presence of apoptotic and necrotic cells. There were no changes on nitric oxide production in the different experimental conditions tested. Understanding this initial response against *Leishmania amazonensis* parasite can be very important to interfere in therapeutics and vaccine strategy. Supported by CNPQ and Millennium Institute.

## IM02 - Involvement of respiratory burst in the apoptotic death of Leishmania guyanensis in murine macrophages

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Murine cutaneous leishmaniasis has different outcomes determined by either the species of *Leishmania* or the mouse strain. In the present work, we show that BALB/c mice, which are incapable of healing *L. major* or *L. amazonensis* lesions, do not develop lesion when infected with *L. guyanensis*. Accordingly, the percentage of macrophages infected *in vitro* with *L. guyanensis*, but not with *L. amazonensis*, decreases dramatically in 96 hs after the infection. Using the TUNEL technique, we observed that in 24 hs after infection, 25% of *L. guyanensis*-infected macrophages had parasites with DNA fragmentation, whereas only 8% of *L. amazonensis*-infected cells had stained parasites. We found around 35 positive *L. guyanensis* amastigotes, as oposed to 9 positive *L. amazonensis* amastigotes per 100 macrophages. These results suggest that L. guyanensis amastigotes die through a mechanism at least similar to apoptosis inside macrophages. Since *L. guyanensis*-infected macrophages do not produce detectable levels of NO, we looked at the ability of *L. guyanensis* to induce the respiratory burst. We found that *L. guyanensis* induces the production of oxygen intermediates by BALB/c macrophages, as determined by chemoluminescence. Specific inhibition of NADPH oxidase impaired the capacity of BALB/c macrophages to eliminate *L. guyanensis*, demonstrating the involvement of the respiratory burst in the parasite death. We also show that $H_2O_2$ is able to induce DNA fragmentation in *L. guyanensis* promastigotes. Our results indicate that *L. guyanensis* dies through a mechanism similar to apoptosis mediated by reactive oxygen intermediates generated in BALB/c macrophages during infection.

## IM03 - In silico and in vitro identification of Leishmania chagasi HSP70 antigenic domains

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Heat shock proteins, a group of evolutionarily conserved proteins, play an essential role both in innate and specific immune responses. This immunostimulatory activity has been assigned to the most divergent, carboxy-terminal region of the molecule, with a number of antigenic determinants indicating a concentration of linear epitopes for B cells. In the present work, we identified the cytoplasmic HSP70 of L.chagasi, by similarity studies, using the ProGeNE (Northeastern Genome Project) EST database. The full length cluster was formed by several clones of different lengths, covering the entire molecule. A novel antigenic region of HSP70 was identified in the median region, 5‘ of the immunodominant EADDRA epitope, presenting reactivity against visceral leishmaniasis patients sera. Recombinant polypeptides containing progressively larger HSP70 sequences were used in an immunoadsorption assay to identify antigenic regions over the full range of the protein. The deduced aminoacid sequences for HSP70 were compared with a large set of similar sequences of trypanosomatids and non - trypanosomatids and divergent regions were clearly associated with epitopes or antigenic regions, defined in this work or elsewhere. This work demonstrates that the association of in silico analysis and in vitro experiments may help elucidate the mechanism of B cell epitope determination and antigenicity of HSP in parasitic and microbial infections. Keywords: Leishmania chagasi; HSP70; B cell epitope; serodiagnosis; visceral leishmaniasis.

## IM04 - BALB/c MACROPHAGES ELIMINATE LEISHMANIA GUYANENSIS IN VITRO, THROUGH A NO-INDEPENDENT MECHANISM.

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We have previously shown that BALB/c mice, which are extremely susceptible to *L. major* or *L. amazonensis* infection, do not develop any sign of lesion when infected with *L. guyanensis*. In addition, the number of intracellular amastigotes dramatically decreases in macrophages infected in vitro with *L. guyanensis*, but not with *L. amazonensis*. Here, we investigate, the mechanism whereby macrophages eliminate *L. guyanensis in vitro* in an attempt to understand the basis of the specific resistance of BALB/c mice to *L. guyanensis*. Initially, to rule out the possibility that macrophages were taking up procyclic promastigotes, known to be incapable to survive in phagolysosomes, peritoneal macrophages were infected with promastigotes derived from cultures of different stages of development. We have shown that, although logarithmic phase promastigotes (around 90% procyclic), are more readily eliminated from the host cell, stationary phase parasites (around 90% metacyclic), are also eliminated. To investigate the involvement of nitric oxide (NO), we either quantified the nitrite produced by infected macrophages (Griess reaction) or inhibited the production of NO (L-NIL) previously to infection. We have verified that macrophages infected with *L. guyanensis* produce no detectable nitric oxide (NO) and that the abolition of NO synthesis does not interfere with the elimination of the amastigotes. Our results demonstrate that BALB/c macrophages are able to eliminate *L. guyanensis* amastigotes *in vitro* in a NO-independent fashion. Our group (Sousa-Franco et al., this meeting) has shown that reactive oxygen intermediates are involved in the elimination of *L. guyanensis* by BALB/c mice.

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## IM05 - Stable oscillatory behaviour between parasite population and adaptive immune response in leishmaniasis.

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In a previous communication, a set of three differential equations was adapted to leishmanial disease particularities (De Almeida, M. C.& H. N. Moreira. 2003. Leishmaniasis: A Dynamical System Approach. Rev Inst Med Trop S Paulo. 45: (Suppl 13)123−124). The set of equations, describing the TH1 arm, TH2 arm and parasite growth, were analysed for conditions of existence and stability of the solutions.
One of main hypotheses was the assumption of a logistic parasite growth curve. Now, the results assume an intrinsic permissiveness of innate immune system on parasite growth. This was done using a parasite exponential growth curve in absence of T Helper response. Before, we obtained the following findings and its possible clinical correlations:1)TH2 and parasite extinction [TH1 cure] 2)TH1 extinction ,TH2 and parasite coexistence [stable TH2 infection], 3)TH2 extinction, TH1 and parasite coexistence [stable TH1 infection], 4)TH1, TH2 and parasite coexistence [stable TH1/TH2 infection]. Geometrically the situations 1) and 2) were characterised as stable nodes and situations 3) and 4) as stable spiral focus. Some predictions of the model were in harmony with experimental data : TH1 cure, stable TH1 infection and stable TH1/TH2 infection. The main new consequences are: 1) absence of stable TH2 infection.In the former analysis we have assumed a strong intrinsic restrictive action of innate immune system on parasite growth which contributed to a stable TH2 infection. 2) A family of periodic orbits in situation 4(stable TH1/TH2 infection), instead of stable spiral focus. Periodic orbits appear to be a more real thing in most biological models of leishmaniasis, describing an oscillatory equilibrium throughout the time among parasite population in the host,TH1 and TH2 responses. One possible interpretation of the logistic and exponential systems could be related to presence or absence of *Lsh* gene in the host.
Acknowledgements
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## IM06 - EVALUATION OF HUMORAL AND CELLULAR IMMUNE RESPONSES IN DOGS IMMUNIZED WITH Leishmania chagasi AMASTIGOTE RECOMBINANT ANTIGENS IN COMBINATION WITH A PLASMID ENCODING RECOMBINANT CANINE IL-12.

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Introduction and Objectives: Zoonotic visceral leishmaniasis is an endemic disease in the Mediterranean basin, Asian countries and South America. Since the domestic dog is the major reservoir of the causative agent, *Leishmania chagasi*, an effective canine vaccine could, in theory, contribute to the control of the disease in both humans beings and dogs. The protective immune response against canine visceral leishmaniasis seems to be a Th1-type cellular response. IL-12 is a potent immunomodulator capable of inducing Th1-type immune response to co-administered antigens. It is described, herein, the immunogenicity for dogs of two *L. chagasi* amastigote recombinant antigens in combination with recombinant canine IL-12 single-chain encoding plasmid. Methods and Results: Two recombinant *L. chagasi* antigens (Lc9 and Lc13) were selected from a cDNA library, using pool of sera of dogs naturally infected with *L. chagasi* and with *Leishmania* specific DTH. The Lc9 and Lc13 proteins used for immunization were produced in *Escherichia coli*. Canine IL-12 was constructed as a single-chain in pcDNA3.1zeo plasmid (pIL-12) and was shown to be biologically active. Groups of dogs were injected with three doses, in three-week intervals, of: i) saline/saponin (3 dogs); ii)

antigens/saponin (4 dogs) or iii) antigens/pIL-12/saponin (3 dogs). Dogs injected with antigens in saponin with or without pIL-12 developed specific humoral immune responses, measured by ELISA, at three weeks after the last immunization. However, there was no difference in cellular immune reponses among the groups, as assessed by specific lymphoproliferative assay around four months after the third immunization. Conclusion: Immunization with Lc9 and Lc13, with pIL-12 or not, in saponin, induced humoral immune responses but fail to promote significant cellular immune responses. The lack of cellular immune response in the animals injected with pIL-12 may be due to the administration of an insufficient amount (800$\mu$g/dose) of plasmid.

## IM07 - A POTENTIAL MODEL OF RESISTANCE TO *L. (L.) AMAZONENSIS* INFECTION

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Description of resistance to *L. (L.) amazonensis* is controversial, strengthening the importance of characterization of an animal model resistant to this *Leishmania* species. The present work focuses on the *L. (L.) amazonensis* infection of two mouse strains, BALB/c and C3H/HePas, considering some cellular immune responses related to infection. Following the *L. (L.) amazonensis* infection of the two mice strains for 10 weeks it was shown that the foot lesions of C3H/HePas mice were significantly lower compared to those exhibited by the BALB/c mice. The histopathology of foot lesions from both mice strains was also evaluated. Thin sections of foot lesions from C3H/HePas mice showed a high inflammatory reaction characteristic of a granulomatous process with presence of epithelioid and plasma cells, frequent neutrophils, and very few infected macrophages. In contrast, in the BALB/c foot lesions there was a predominance of macrophages harboring a large number of amastigotes and presence of very few lymphocytes and neutrophils. In vitro *L. (L.) amazonensis* infection of bone marrow macrophages isolated from BALB/c and C3H/HePas mice did not show significant differences in the phagocytic indexes between the two strains, indicating that the resistance of the C3H/HePas strain to *L. (L.) amazonensis* is not related to "primary"leishmanicidal capacity of macrophages. Results from lymphoproliferation induced by a recombinant cysteine proteinase from *L. (L.) amazonensis*, Lacys24, showed that the resistance of the C3H/HePas could be related to a defferential recognition of parasite antigens by lymphocytes. In addition, the presence of neutrophils even after nine weeks of infection in foot lesions from C3H/HePas suggests a possible role for these cells in the infection control. The present work opens perspectives to use the C3H/HePas strain as a resistance model to *L. (L.) amazonensis* in order to understand the leishmanicidal mechanism against these parasites developed by these animals.

Supported by FAPESP

## IM08 - Development of a natural model of infection using *Leishmania braziliensis*

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We investigated the course of infection with Leishmania braziliensis (MHOM/BR/01/BA788) by intradermal injection of parasites in BALB/c mice. Using this approach, we observed that parasite expansion was accompanied by lesion development at the ear dermis. Lesions were similar to those observed in cutaneous leishmaniasis patients, (ie. nodular and ulcerated in the center) and, beyond four weeks post infection, total lesion involution was observed as seen by the presence of a scar. Histopathological analysis of infected ears showed the presence of, initially, a focal infiltrate consisting of mononuclear cells (lymphocytes and monocytes), neutrophils and few parasites. At the peak of lesion development, infected macrophages predominated. Thereafter, a weak infiltrate consisting of histiocytes, plasma cells, neutrophils and dermal fibrosis was observed with the absence of parasites. Regarding the draining lymph nodes, parasites could be detected throughout the infection period at a constant level. Measurement of intra cellular cytokine response by draining lymph nodes cells showed that, two weeks post infection, there was an up regulation in IFN-gama, IL-4, IL-5 and IL-10 production by both CD4+ and CD8+ T cells. Later, cytokine expression decreased paralleled by lesion regression, with the exception of IL-10. We also evaluated chemokine expression both at the infection site and at the draining lymph nodes. Results show the up regulation in both monocyte/macrophage and granulocyte-recruiting chemokines confirming the histopathological findings. Leishmania inoculation in the ear dermis more closely resembles the natural infection and, similarly to the footpad infection model, BALB/c mice are resistant to L. braziliensis infection due to the development of a predominant Th1-type immune response. However, in this model, parasites are able to persist within draining lymph nodes of infected mice regardless of the development of a protective immune response. We are presently investigating whether this persistence is related to regulatory T cells. Supported by: CNPq, PAPES/FIOCRUZ, FAPESB, TMRC-NIH

## IM09 - A RECOMBINANT CYSTEINE PROTEINASE FROM *LEISHMANIA (L.) CHAGASI* IMPLICATED TO DOG T CELL RESPONSES.

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The present work evaluates the lymphoproliferative responses elicited by a recombinant protein produced by expression of the gene encoding a cysteine proteinase of 30 kDa from *Leishmania (L.) chagasi*, rLdccys1. These responses were determined in peripheral blood mononuclear lymphocytes from naturally infected dogs presenting several clinical signs, living in Teresina, the capital of the Piauí state, Brazil, an endemic region of visceral leishmaniasis. The recombinant antigen elicited higher T lymphocyte responses in dogs presenting asymptomatic and oligosymptomatic visceral leishmaniasis compared to those observed in symptomatic animals. Lymphokine analysis showed a predominance of IFN-$\gamma$ in the lymphocyte supernatants from asymptomatic dogs, whereas lymphocytes from symptomatic animals released significant levels of IL-4 and IL-10. Intermediary values of IFN-$\gamma$ and IL-10 were observed in lymphocyte supernatants from oligosymptomatic patients. A correlation between oxide nitric release and IFN-$\gamma$ secretion was also observed in the supernatants of dog lymphocytes stimulated by rLdccys1. These results show that the recombinant cysteine proteinase from *Leishmania (L.) chagasi* is able to induce and discriminate cellular responses in dogs naturally infected with *Leishmania (L.) chagasi*, opening perspectives to test this recombinant antigen in protection studies in endemic regions of canine visceral leishmaniasis. Supported by Fundação de Amparo à Pesquisa do Estado de São Paulo-FAPESP and Faculdade de Saúde, Ciências Humanas e Tecnológicas do Piauí-NOVAFAPI.

## IM10 - Oral immunization with irradiated tachyzoites of *Toxoplasma gondii*: Analysis of IgG subclasses in serum and brain histopathology in immunized C57BL/6j mice

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Toxoplasmosis, a prevalent widespread infection in man and animals, occurs mainly through ingestion of water and food contaminated with oocyst from cat feces. There is no vaccine but radiation sterilized tachyzoites (RST) parenterally administered induced significant protection. We study RST by oral route, looking for serum IgG subclasses and quantitative protection studies. RST was RH strain tachyzoites irradiated with 255Gy and stored in liquid nitrogen. Mice were immunized biweekly with $10^7$ RST by oral route, suspended in milk and/or aluminum hydroxide. Specific ELISA for IgG1, IgG2a and IgG2b detection was performed in weekly blood samples during and after immunization, with challenge with 10 cysts of ME49 strain p.o. Protection was determined at the 30th day in brain by cyst counting and histological analysis. Parenterally immunized and infected animals presented high levels of all IgG subclasses. P.O. immunized groups presented low levels of IgG2b, similar to controls, but the other isotypes were easily detected in all groups. When challenged, all imunized groups presented low levels of cysts in brains, but without death or clinical signs. All mice immunized presented significant protection, compared to controls (P0.001), with better protection i.p. and aluminum hydroxide groups. Other protocols provided partial protection (P0,05) with less 500 cysts than controls, with confirmation by brain histology. The best protection was related to low levels of serum IgG2a, reported as a marker of Th1 immune response. The effect of aluminum hydroxide was could be related to a buffering effect in gastric juice, allowing RST survival, while milk association can be insufficient to block the peptic digestion or competition among milk protein and *T.gondii* antigens. All these data provide insights in oral immunization schedules for toxoplasmosis prevention, allowing new studies that could results immunizing baits for widespread enviromental vaccination of free living animals.
This work was supported by FAPESP (99/04926-6), LIMHCFMUSP-49, CNPq and CAPES

## IM11 - Laboratory artificial lethal challenge could induce misinterpretation of the protection induced by *Toxoplasma* vaccines

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Toxoplasmosis is mostly asymptomatic in humans and animals, but 1% suffers with eye involvement and visual losses, with deaths in immuno-compromised people and in fetuses when acute disease occurs during pregnancy. There is no commercial vaccine for human toxoplasmosis, with several reports using recombinant proteins, parasite purified proteins or radiation sterilized parasites (RST). The latter presented good results, similar to chronic disease, with low cysts counts in brains of immunized mice in natural infection models, but shows feeble protection against huge artificial challenges with RH tachyzoites. We study the quantitative protection in-

duced by ET against artificial lethal load of cysts. Tachyzoites of RH strain was sterilized with 255Gy (60-cobalt) radiation and groups of C57Bl/6j mice were immunized (i.p.) with three doses of $10^7$ RST at biweekly intervals. A significant increase of antibody anti-*T.gondii* was detected after three doses. Those animals were challenged (v.o.) with 100 ME-49 cysts. All control mice died after two weeks of the challenge, but immunized mice presented a lower mortality (40%) with all survivors presenting cysts in brains. Brain cyst counts were similar to those found in brains of mice challenged with 10 cysts by oral route (a non lethal dose). Those data shows that the immunization with RST results in a protective effect that could be insufficient for artificial challenge, that exceed the immune response capacity to control a parasite. Careful choice of the challenge must be as similar as possible to natural infection, avoiding artificial huge loads, easily obtained at laboratory level. All efforts must be performed in order to quantify the infection in animals. The vaccine testing design must include several approaches of experimental infections, allowing critical evaluation of their results, avoiding misinterpretation of failures of promising candidates.

This work was supported by LIMHCFMUSP-49, CAPES and CNPq(141404/2004-3).

## IM12 - Lymphoproliferative response of *Leishmania (L.) chagasi* experimentally infected dogs of an immunotherapy assay with Leishmune$^R$ vaccine.

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The immunotherapic effect of FML-QuilA or FML-saponin R vaccines was recently proven against experimental and natural canine leishmaniasis. A strong protective response was obtained with positive DTH, normal levels of CD4+ T cells and CD21+ B cells, high levels of CD8+ lymphocytes and absence of parasite in bone marrow. At day 150, antibodies were detected in the FMLELISA assays of all dog samples. In the present work, we analyzed the T-cell reactivity to leishmanial antigens of 25 dogs infected with 2x $10^8$ amastigotes of *Leishmania (L.) chagasi*. The lymphoproliferative response (LPR) of PBMC was performed at day 180 (before vaccination). PBMC were separated over a Ficoll-hypaque gradient. The cells (4x$10^5$ per well) were stimulated with f/t lysate of stationary phase ($10^6$ promastigotes per well) of *L. (L.) braziliensis*, *L. (L.) chagasi* and *L. (L.) donovani* and incubated at 37°C, 5% $CO_2$ for days. Sixteen hours before harvesting, 1 $\mu$Ci of [$^3$H] , and the radioactivity uptake was measured in a scintillation Beta-counter. All dogs showed positive responses for the three antigens: mean averages ± SD (stimulation index): *L.(L.) braziliensis* (2.27±4.57), *L.(L.) chagasi* and (4.61±14.70) and *L. (L.) donovani* (3.63±13.63). However, 15 dogs developed their highest response against *L.(L.) chagasi*, 5 dogs against *L.(L.) braziliensis* and 3 dogs against *L. (L.) donovani* suggesting a mild species-specificity recognition. The analysis of the proliferative response of vaccinates versus control animals in now in progress.

Support: CNPQ; FAPERJ; RHAE-CNPQ; FIOCRUZ, Fort Dodge Animal Health Brazil and USA.

## IM13 - Phase I safety and immunogenicity trial of Leishmune$^R$ in dogs of an endemic area

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A group of 482 asymptomatic dogs from São Paulo and Minas Gerais endemic areas of canine visceral leishmaniasis was vaccinated with Leishmune$^R$ (1.5mg of FML and 0.5mg of Saponin R). Animals received three sc doses with 20-30 days intervals and one annual boost. For ethical reasons, veterinarians were not able to keep a control population of untreated and exposed dog. With the aim of comparison then, we also show the data collected from a control exposed dog population of Jardim Progresso, Natal, RN, Brazil where human and canine kala-azar is also endemic. Among 482 vaccinated dogs only 432 were seronegative in the FML-ELISA assay at D0 and asymptomatic. Strong seroconversion was detected after complete vaccination (98.2%). By this time, only 15% of the controls developed anti-FML antibodies. Regarding the clinical development of the disease, at month 7 after vaccination, only 0.92% of the vaccinated dogs showed clinical or parasitological signs of kala-azar while 5.44% of the untreated controls showed kala-azar symptoms ($x^2$= 149.44; p¡0.001). This difference could be even more pronounced since the untreated controls are submitted to regular serological epidemiological survey which removes for sacrifice the seropositive reservoirs. Also at month 7, the intradermal reaction of vaccinees was positive in 59% of the animals. At month 9, differences between vaccinees and controls are more pronounced. Indeed, while no kala-azar obits were found among vaccinees, 7% of serologically and clinically confirmed and 8% of clinically suggestive obits were scored in the control group (p¡0.001). Also, 13% of symptomatic cases were detected among controls and only 2.4% among vaccines (p¡0.001). Our results indicate the strong protective prophylactic effect of Leishmune$^R$ in seronegative dogs of endemic areas. The Leishmune$^R$ industrial vaccine reproduces the previously reported protective effect of the FML saponin vaccine. Support: CNPQ; FAPERJ; RHAE-CNPQ; Fort Dodge Animal Health Brazil and USA.

## IM14 - Toxoplasmosis diagnosis by saliva ELISA

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*Toxoplasma gondii* is an parasite widely distributed in humans and animals, being the main zoonotic cause of morbidity, but with low mortality only in selected groups. The diagnosis is usually performed by serological tests, with demonstration of anti-*T.gondii* antibodies IgG and IgM, with infection period determined by IgG avidity. The diagnosis of acute infection for screening of congenital toxoplasmosis is usually performed by selecting anti *T.gondii* IgM positive mothers during prenatal care, as an index of acute infection, but with low efficiency and high frequency of unneeded treatments. The antenatal selection of mothers at risk of acute infection could be an alternative but must be performed just after the fertile age, during high school. These populational study must need a precise, non-invasive and inexpensive tool for detecting Toxoplasma infection and we devised a anti *T.gondii* human IgG ELISA using saliva as antibody source. Saliva samples, collected by tooth brushing with cotton swabs, were recovered from the swabs by centrifugation im microfuge after washing with 500 ul of borate buffer. Saliva was compared with simultaneously collected blood samples in an ELISA standardized using positive sera tested with indirect immunofluorescence assay. Microplates adsorbed with saline extract of RH strain tachyzoites from experimentaly infected mice were incubated with serially diluted saliva and serum, with bound IgG revealed with specific peroxidase conjugate. The cut-off of undiluted saliva from negative patients was 0.255. There are a good agreement between saliva and blood in this reaction with sensitivity of 100% and specificity of 75% in our sample. The data also presented a clear dose response in saliva diluted samples. This test may be a good auxiliary role in diagnosis of toxoplasmosis, especially in children, because is a non-invasive alternative, with easy sampling method of collecting, reducing costs and risks related to blood processing. Miriam de Souza Macre is folowed by CAPES and LIM 49 HCFMUSP.

## IM15 - USE OF A GENE ENCODING THE CYSTEINE PROTEINASE OF 30 kDA FROM *L. (L.) CHAGASI*, *LDCCYS1*, FOR VACCINATION OF BALB/C MICE AGAINST HOMOLOGOUS INFECTION.

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This works describes the cloning and expression of the gene encoding the cysteine proteinase of 30 kDa from *L. (L.) chagasi*, *Ldccys1*, and use of the recombinant DNA in active immunization of BALB/c mice. The *Ldccys1* gene was cloned by PCR amplification using genomic DNA from *L. (L.) chagasi* amastigotes and primers corresponding to the open reading frame sequence of the *Ldccys1* gene published in GeneBank. A product of 1.3 kb (clone 1.3) was amplified and sequence analysis of the clone showed a high identity to sequences of other *Leishmania* cysteine proteinase genes as *L. (L.) infantum cpb*, *L. (L.) mexicana cpb18*, and *L. (L.) pifanoi Lpcys2*. Analysis of the predicted amino acid sequence of Ldccys1 showed the presence of all conserved residues characteristic of a cysteine proteinase and previously identified in the *Ldccys1* gene sequence. The *Ldccys1* gene was also subcloned in the pcDNA3 vector and injected in BALB/c by the intramuscular route. After immunization the animals presented a strong humoral response with production of IgG1, IgG2a and IgG2b isotypes. Spleen lymphocytes from these animals did not display proliferative responses when stimulated with *L. (L.) chagasi* antigens, including rLdccys1, although they produced IFN-$\gamma$, IL-4 and IL-10, indicating that vaccination with the *Ldccys1* gene induced a mixed Th1/Th2 response. Challenge with *L. (L.) chagasi* amastigotes resulted in a significant decrease in the parasite burden of animals immunized with the *Ldccys1* gene when compared to those which received PBS. Nevertheless, there was no difference in parasite burdens of animals immunized with empty pcDNA3 or *Ldccys1*/pcDNA3 plasmid. These results led us to search for new vectors to clone the *Ldccys1* gene for use in immunization schedules which are currently in progress.
Supported by FAPESP and CAPES.

## IM16 - The ROC curve test for antigen screening to be used in ELISA for American Cutaneous Leishmaniasis caused by *Leishmania braziliensis*

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American Cutaneous Leishmaniasis (ACL) is caused by different *Leishmania* species and a precise diagnosis can be performed by molecular approach on the isolated parasite from the infection. In this contest, serological tests are preferred methods to complement the diagnosis of ACL, in spite of its low diagnostic value. This work shows the performance of different antigens, evaluated by test receiver operating characteristic curves (ROC) in order to screening the best antigen to be used in ELISA for ACL by *L.braziliensis*, in Rio de Janeiro (RJ). ELISA tests were performed with crude antigen (500 ng/well) from *L.braziliensis*, isolated from RJ, *L.amazonensis* and *L.major* promastigotes. The panel used in those tests consisted of 347 samples from men and women (18 until 65 year old). In this panel, sera samples from patients with clinical and parasitological confir-

mation of ACL (n=65), from individuals with other clinical syndromes (n=160), from individuals without leishmaniasis but living in endemic area (n=22) and from blood donors (n=100) were evaluated. All data obtained were analyzed statistically using Medcalc software. Three groups were formed according to the results of an exploratory analysis of the antigen in an area under the curve (AUC) of ROC curves. The AUC for the *L.braziliensis*, *L.amazonensis* and *L.major* antigen preparation used to sensitize plates in wich the assay correctly identified the patients were respectively, 79.1% (AUR=0.892), 75.8% (AUR=0.871) and 47.8% (AUR=0.613) of the patients. These data indicates that those antigens derived from *Leishmania* strains that are causing disease in a given area are most useful to diagnose leishmaniasis in such endemic area. Since the epidemiological incidence of ACL in Rio de Janeiro is predominantely caused by *L.braziliensis* with progressive increase in the last years, these results may refer to the use of homologous antigen in clinical and epidemiological studies and control programs. Supported by CAPES/FAPERJ/CNPq.

## IM17 - Hyperbaric oxygen therapy in the treatment of mice infected by *Leishmania amazonensis*

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Hyperbaric oxygen (HBO) therapy has been shown to increase both systemic and wound tissue oxygen levels and to assist as adjuvant to surgery and antibiotics to numerous soft-tissue infections. However, there are few studies conducted to stabelish the effect of HBO therapy in parasitic diseases. In the present study we analysed the effect of HBO therapy during *Leishmania amazonensis* infection in susceptible BALB/c mice. We also compared the effect of the reference drug Glucantime to the of HBO. Animals were exposed to HBO (0.5 ATA/min to a pressure of 2.5 ATA; 100% $O_2$) for 1 h before and 2 h imediately after the infection (1 h/day to 2.5 ATA; 100% $O_2$). Alternativaly mice were exposed to HBO during 3 or 20 days after infection. Both HBO treatments reduced significantly the susceptibility to infection of BALB/c mice as defined by decreased size of infection footpads and 4-fold, 5-fold reduced burdens at 3 and 20 days after infection, respectivaly. HBO treatment led to attenuate visceralization in spleen and liver and produces an effect similar to that of treatment with Glucantime (100 mg/kg/day during 20 days) in infected BALB/c mice. Hystopathological analyses revealed a mixed cellular population infiltrating the tissue, few parasitized macrophages and conserved epidermis tick and glandular structures in the lesions of HBO treated mice, similar to lesions of Glucantime treated mice. In contrast, footpad tissue from non-treated BALB/c mice showed macrophages contaning numerous amastigotes within parasitophorous vacuoles and replacing the normal tissue. The protective *in vivo* effect of HBO during murine leishmaniasis encourage further studies of this treatment to combat parasitic infections. Supported by FAPESP, CAPES and CNPq.

## IM18 - Hypoxia reduces *Leishmania* infection in macrophages and modulates the expresion of 70 kDa heat shock protein

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Hypoxia, a micro environmental factor present in diseased tissues, has been recognized as a specific metabolic stimulus or a signal of cellular response. Experimental hypoxia has been reported to induce adaptation in macrophages such as differential migration, elevation of pro-inflammatory cytokines and glycolytic enzyme activities, and decreased phagocytosis of inert particles. In this study we demonstrate that, although exposure to hypoxia (5% $O_2$, 5% $CO_2$ and balanced $N_2$) did not change macrophage viability, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cleavage and proliferation, it reduced significantly 70 kDa heat shock protein (HSP70) expression, which was restored to pre-hypoxia levels after reoxygenation. The influence of low oxygen tension on macrophage functional activity was also studied, i.e. the ability of these cells to maintain or resist infection by a microorganism. We demonstrated that macrophages from two different sources (a murine cell line and a primary cell) exposed to hypoxia were efficiently infected with *Leishmania amazonensis*, but after 24 h showed a reduction of the percentage of infected cells and of the number of intracellular parasites per macrophage, indicating that hypoxia induced macrophages to kill intracellular parasites. These results support the notion that hypoxia, a micro environmental factor, can modulate macrophage expression and functional activity. Supported by FAPESP, CNPq and CAPES.

## IM19 - The *in vitro* susceptibility of *Leishmania amazonensis* to hyperbaric oxygen

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Hyperbaric oxygen (HBO) is 100% oxygen administrated at elevated atmosphere pressure. The HBO therapy is applied for patients with local deficient perfusion such as soft-tissue infections. The cellular production of reactive oxygen species, which increases during exposure to hyperoxia and HBO is probably the major factor of the oxygen toxicity. Exposure to elevated oxygen tensions affect viability of some parasites such as *Giardia duodenales*, *Entamoeba hystolitica*, *Pneumoscystis carinii* and *Schistosoma mansoni*. Since *Leishmania* exhibits antioxidant defense, but is lysed by reactive oxygen species, we evaluated the effcts of HBO exposure in both *L. amazonensis* life stages (promastigotes and amastigotes) and on macrophage cultures infected with the parasite. HBO treatment protocols, which can be tolerated by humans and animals, induced irreversible metabolic

damage and affected parasite morphology, growth, ability to transform and mitochondria activity which was evaluated by MTT test. Electron microscopy demonstrated an increase in cytoplasmic lipids bodies in promastigotes forms. The observation that the antioxidant N-acetylcysteine prevents some of these deleterious effects indicated an involvement of oxidative stress during parasite HBO exposure. In addition, HBO exposed *L. amazonensis*-infected macrophage cultures showed reduction of percentage of infected cells and of the number of intracellular parasites per cell. Thus, the demonstration that HBO, a therapy used in the managementof different diseases, is toxic for both *L. amazonensis* life stages and can alter susceptibility to the infection, encourages further studies of this therapy in animal models of *Leishmania* infection. Suported by CNPq, CAPES, FAPESP and FAEP.

## IM20 - Trypomastigotes is the only *Trypanosoma cruzi* form that exposes phosphatidylserine

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*Trypanosoma cruzi*, the agent of Chagas diseases, infects more than 10 million individuals in Latin America. This parasite presents three different forms: amastigotes, epimastigotes and trypomastigotes. It has been shown that *Leishmania amazonensis* exposes phosphatidylserine (PS) on the cell surface, which is important for its evasion mechanism. We have recently demonstrated that tachyzoites of *Toxoplasma gondii* exposes PS, which is involved in the down modulation of nitric oxide of activated macrophages. The aim of the present work was to determine if PS is also exposed on the different forms of *T. cruzi*. The Y strain and the Dm28 clone of *T. cruzi* were used. Epimastigotes were obtained axenically after 6 day in culture. Trypomastigotes were purified from: a) blood harvested from mice, b) the supernatant of infected Vero cells (Y strain), c) after metacyclogenesis (metacyclic trypomastigotes) in chemically defined medium (Dm28c). Trypomastigotes from infected Vero cells were also incubated in LIT medium for epimastigote transformation. Amastigotes were obtained by amastigogenesis in chemically defined medium (Dm28c). PS exposure of the three different forms was analyzed by flow cytometry after annexin-V-FITC and propidium iodide labeling. Trypomastigotes, independently of the source, exposed PS. However, epimastigotes and amastigotes were negative. Incubation of trypomastigotes in LIT medium for 24 hours abolished PS exposure. These results indicate that PS might also be used by *T. cruzi* to evade innate immunity. It also demonstrates that PS exposure might by a common feature of obligate intracellular parasites that have to deal with activated macrophages.
Supported by: FAPERJ, CNPq, Fiocruz, PRONEX.

## IM21 - HIV-1 INFECTION AND THE HIV-1 TRANSACTIVATOR TAT PROTEIN INCREASE BLASTOCRITHIDIA CULICIS SURVIVAL IN MACROPHAGES.

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Infections by monoxenous trypanosomatids were described in HIV-positive patients, causing a leishmaniasis-like syndrome. These trypanosomatids, found mainly in insects, were normally non-pathogenic for mammals. With the emergency of monoxenous trypanosomatids as opportunistic pathogens in immunocompromised patients, we decided to study the co-infection of Blastocrithidia culicis and HIV-1, in vitro. Thus, human monocyte-derived macrophages (MDM) were infected with a R5-tropic HIV-1 isolate using 10 ng/ml of p24 antigen. After 10 of days infection, B.culicis was added to the HIV-infected MDMs at a 1:10 ratio, and cultures were kept for 48h at 35oC. Viral and parasite replication were evaluated by p24 ELISA assay and endocytic index, respectively. B. culicis-infected murine macrophages were also exposed to the HIV-1 transactivator protein Tat, and the levels of TGF-b in culture supernatants were evaluated, and protozoa growth was measured as well. We found that HIV-1 infection increased 2 times the B.culicis survival in MDM. To further analyse whether a HIV-1 component could mediate this effect, we treated mouse macrophages infected with B.culicis with HIV-1 Tat protein. Treatment with 100 ng/ml of this protein increased the protozoan growth 9 times after 24h, and 4 times after 48h, relative to control. Since Tat stimulates the production of TGF-b by macrophages, we also investigated the role of this cytokine in B.culicis infected-mouse macrophages. Addition of TGF-b at 10 ng/ml increased 8 times the endocytic index after 24h infection, relative to control. We found that HIV-1 Tat protein and TGF-b upregulate the growth of B.culicis in macrophages. Our results show for the first time that HIV-1 infection changes an otherwise non-pathogenic B.culicis to an opportunistic pathogen and this mechanism could be mediated by HIV-1 Tat protein through TGF-beta production.
Supported by: CNPq, PAPES/Fiocruz. Faperj.

## IM22 - HIV-1 TAT PROTEIN EXACERBATES LEISHMANIA GROWTH IN HUMAN MACROPHAGES

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Individuals with HIV-1 usually succumb to co-infections, commonly associated with the decline in the number of CD4+ T cells. In this context, Leishmania appears as a frequent opportunistic pathogen, especially in the Mediterranean countries. It has been demonstrated that HIV-1 modulates Leishmania growth in human macrophages, but the mechanisms are poorly understood. In this study, we investigated whether Tat, an HIV-1 protein that acts in the transcription of the viral genome, and is found in the serum of AIDS patients, could regulate the replication of Leishmania in primary human macrophages. Human macrophages were co-infected by HIV-1 and Leishmania amazonensis, and viral and parasite replication were evaluated by p24 ELISA assay and phagocytic index, respectively. Leishmania-infected macrophages were alternatively exposed to Tat, and the parasite growth was measured after 3 days. In some assays, anti-TGF-b or the COX-2 inhibitor celecoxib were added to Leishmania-infected macrophage simultaneously to Tat. We found that HIV-1 infection augmented Leishmania growth, varying from 20-60%. In macrophages infected with Leishmania and exposed to HIV-1 Tat, Leishmania replication increased according to a dose-dependent manner of Tat, reaching the peak of 2 fold-increase with 80 ng/mL of Tat. To analyze a possible mediator involved in this process, macrophages were treated with Tat in the presence of anti-TGF-b or celecoxib. Both treatments, independently, suppressed no more than 50% of the enhancement effect mediated by Tat. Therefore, we are currently investigating whether PGE2 and TGF-b could act in synergy in the exacerbation of Leishmania replication. Our results suggest that HIV-1 Tat protein enhances Leishmania growth through PGE2 and TGF-beta production. We show here for the first time that a component of HIV-1 induces replication of Leishmania within macrophages, suggesting that the HIV-1-induced immunosuppresion does not totally explain the development of leishmaniasis in HIV-1-infected patients.
PAPES/Fiocruz, Faperj, CNPq

## IM23 - INFLUENCE OF SALIVA FROM *Lutzomyia longipalpis* IN INITIAL EVENTS OF INFECTION BY *Leishmania chagasi*

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**Introduction and objectives:** Salivary components from *Lutzomyia longipalpis* influence immunity against the protozoa *Leishmania*. Interactions between the parasite, APC and lymphocytes may determine the outcome of the infection. Thefore we evaluated the initial immune response against *Leishmania chagasi* from PBMC normal donors using an in vitro priming system. We tested the capacity of the *L. chagasi* alone or simultaneously with salivary sonicated gland to stimulate the cells in the absence of $CD4^+$ or $CD8^+$ T cells. **Methods and results:** Briefly, PBMC were separated by ficoll gradient and following were overtake through magnetic column with anti CD4 and anti CD8 antibodies, the negative fraction collected and cultured in 24 well plates at $5x10^6$/mL and subsequently exposed to *Leishmania chagasi*. Promastigotes stationary phase ($1x10^6$/mL) plus 2 pair/mL of *L. longipalpis* salivary gland. After 48 or 72 hours, supernatants were harvested for ELISA and cytokine quantification. This system allowed us to detected cytokines, which can be modulated by the parasite and salivary gland sonicate. We found that *L. chagasi* plus SGS stimulated only a weak IFN-$\gamma$ production by whole PBMC, whereas, in the population depleted from $CD8^+$, a higher production of IFN-$\gamma$ was observed. Moreover IL-10 production in the absence of CD8 cells was less evident, and undetectable in the whole PBMC. **Conclusions:** The phlebotomine saliva components appear to affect the functions of the main cells involved in immune response to *L. chagasi*, altering cytokine profile, which are important to drive cell differentiation, and in this sense a protective immune response against *Leishmania*. Actually we are looking for different markers of activation and trying to identify the influence of saliva in the immune response against *Leishmania chagasi* .
Supported by: FAPESB and TMRC-NIH

## IM24 - Generation of dendritic cells from human peripheral blood and infection by *Leishmania amazonensis*

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Dendritic cells (DCs) are the most potent antigen-presenting cells. DCs can be differentiated by culturing monocytes of peripheral blood in medium with different cytokines. In mammalian hosts, *Leishmania* are obligate intracellular parasites that are phagocytosed by macrophages and DCs. In this study, we examined i) several protocols for generating reasonable number of dendritic cells from human peripheral blood monocyte cells (PBMC) and ii) the ability of these DCs to internalise different stages of *L. amazonensis*. DCs were generated from PBMC and cultivated in Iscove´s medium supplemented with combinations of rhGM-CSF, rhIL-4, supernatants of H28 cells (IL-4 source) and supernatants of 5637 cells (GM-CSF source). PBMC were cultured in 24-well plates containing glass and/or 75 $cm^2$ flasks (0,5 x $10^6$

cells/mL). Non adherent cells were removed and adherent cells were cultured for 7 to 10 days at 37°C under 5% $CO_2$. Phenotypical and morphological analysis were performed by flow cytometry and optical microscopy. The results showed that, among protocols tested, cells cultivated with rhIL-4 at 250 ng/mL plus rhGM-CSF at 50 ng/mL during 7 days was the most effective condition of generating morphologic and phenotypic DC-like cells. DCs were HLA-DR$^+$, CD80$^+$, CD86$^+$, CD83$^+$, CD1a$^+$ and negative for CD3, CD14 and CD19. Human DCs were incubated with *L. amazonensis* for 24 h, i.e., amastigotes from infected nude and infected BALB/c mice, non-opsonised and opsonised parasite per infected DC were recorded after staining and microscopic examination. Our results demonstrated that DCs from PBMC cultured in the condition selected above are able to internalise both amastigotes and promastigotes of *L. amazonensis* and to mantain the parasite infection. These findings provide a tool to study phenotypical and functional alterations of human DCs during *L. amazonensis* infection. Supported by FAPESP and CNPq.

## IM25 - Histopathological study of Chagas disease in newborn and adult C57BL/10 mice experimentally infected

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The Chagas disease is a protozoan infection caused by the flagellate *Trypanosoma (Schizotrypanum) cruzi*, widespread in the American Continent chiefly among small wild mammals (enzootic sylvatic cycle). Human Chagas disease constitutes a more recent situation, in which bio-ecological and socioeconomic factors leave rural poor populations of South and Central America in contact with the sylvatic cycle, where the parasite is transmitted by natural vectors of the infection. From the Public Health standpoint, the importance of Chagas disease remains correlated to this so-called "domestic"cycle, not only because millions of human beings are involved but also because all the available control measures are directed against it. Trypomastigotes, the infective forms, are capable of invading and replicating in different cell types, a property that gives the parasite access *in vivo* to privileged sites for survival. The most affected group are children, especially black one, which develop a severe symptomatic acute illness with encephalitis and myocarditis. The aim of the present work was to analyse age as a resistance factor in *T. cruzi* infection in newborn and adult C57BL/10 mice intraperitonially infected with $10^4$ Y strain *T. cruzi* trypomastigotes. Parasitaemia, mortality and histopathology were analyse. Newborn mice presented amastigotes and inflammatory infiltrate in several organs. Adult mice presented fewer parasites in the majority of the organs, but inflammatory infiltrate was more exacerbated. These results show that a tissular invasion by *T. cruzi* followed by an inflammatory infiltrate more or less severe leads animals to death. As newborn mice are not immunologicaly competent, they develop a more severe infection, with a more exacerbated parasitaemia, tissular invasion and mortality.

## IM26 - Role of TNF-a Receptor 1 (TNFRp55) in the delayed type hypersensitivity in response to a formalin-treated Leishmania major

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Tumor necrosis factor (TNF) is an important cytokine for the development of delayed type hypersensitivity (DTH). It mediates capillary permeability; expression of adhesion molecules and up-regulates chemokine expression. In order to investigate the role of TNF-a receptor 1 (TNFRp55) on DTH, C57BL/6 and TNFRp55-/- mice were infected with L. major in the right footpad and after 6 weeks, injected with 107 formalin-treated parasites in the left footpad. The size of the response was measured at 6, 24, 48, 96 hours and 7 days after injection of antigen. While C57BL/6 presented a positive DTH, TNFRp55-/- mice presented no detectable response. Hence, we determined the expression of RANTES, MCP-1, MCP-5, CRG-2, MIG and KC by RT-PCR at the DTH site. We found that C57BL/6 expressed higher levels of RANTES, MCP-5 and MCP-1 than TNFRp55-/-mice at 48 hours, which was the peak of DTH. After that, the expression of these chemokines was down-regulated. TNFRp55-/- mice showed a late and moderate increase in chemokine expression around 96 hours after antigen injection. The late up-regulation of RANTES expression did not induce significant changes in the footpad size and only a mild inflammatory infiltrate could be observed. These results suggest that the kinetics of inflammation is altered in TNFRp55-/- mice rendering a delayed and insufficient response during DTH. To investigate if the results observed were due to a deficient migration of memory lymphocytes, we treated naïve mice with carrageenan in the footpad. C57BL/6 mice presented typical lesions that peaked around 48 hours post-injection and subsequent resolution. In spite of presenting a similar kinetic of lesion development, smaller lesion sizes were found in TNFRp55-/- mice, as well as a delay in the resolution of the inflammatory infiltrate. Our studies suggest that TNFRp55 mediates the migration of cells to the site of inflammation, by modulating chemokine expression.

## IM27 - INTRA-SPECIES PARASITE DETERMINANTS INFLUENCE THE OUTCOME OF *Leishmania(Viannia)* braziliensis CUTANEOUS INFECTION IN A PRIMATE (*Macaca mulatta*) MODEL

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Taxonomic studies have shown genetic polymorphism in natural populations of *L. (V.) braziliensis*, but the risk factors for distinct clinical outcome are still to be determined. As an approach to understanding how the intra-species parasite variability could have a relationship with clinical pleomorphism of the disease, we examined the differential response of rhesus monkeys to infection with distinct *L. (V.) braziliensis* genotypes. All of the primates (6 monkeys/group) inoculated intradermally in the forearm with *L. (V.) braziliensis* isolates from patients with either CL (A), or disseminated CL (B), or mucosal disease (C) developed chronic ulcerative CL that persisted as long as 26 months. The average (maximum) size of the skin lesions was not significantly different when compared among groups, but certain clinical phenotypes (development of metastatic skin and/or mucosal lesions, which persisted until the end of the observation period) were most often observed in monkeys of group B (n = 4). The higher virulence of the latter pathogen was not apparently associated with failure to induce parasite-specific T-cell responses (as detected by measuring DTH reaction, *in vitro* lymphocyte proliferation, and gamma interferon production) in experimental animals. These findings provided evidence for the participation of pathogen virulence in the evolution of CL. Other influences (such as the host-pathogen interactions that influence the granulomatous response) may also affect the type and scale of the local cellular response acting to limit parasite multiplication in the skin lesions. This primate model could be useful for understanding the infection-induced immunopathology associated with such pathogens. Supported by grants from FIOCRUZ, PRONEX-CNPq; and FAPERJ (BRAZIL).

## IM28 - *Toxoplasma gondii* modulate NF-kappaB translocation in activated macrophages by IkappaB alpha

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*Toxoplasma gondii* is an obligate intracellular protozoan, which causes toxoplasmosis. The activation of macrophages with interferon-gamma (IFN-gamma) and lipopolysaccharide (LPS) induce the translocation of NF-kappaB to the nucleus of macrophages up-regulating the transcription of important genes such as the nitric oxide (NO) machinery. IkappaB-beta functions as a classical cytoplasmic inhibitor of NF-kappaB by avoiding nuclear translocation. Moreover, IkappaB-alpha turns off nuclear NF-kappaB and translocated it to the cytoplasm. Our group has demonstrated that *T. gondii* infection can deactivated macrophages by exposing phosphatidylserine that induces TGF-beta production, inhibiting NO production. It has also been show that NF-kappaB does not translocated to the nucleus of macrophages infected by *T. gondii*. To understand how *T. gondii* infection controls NF-kappaB translocation, mice peritoneal macrophages were seeded over coverslips, cultured in DMEM with 5 % fetal bovine serum and activated with IFN-gamma and LPS. Tachyzoites, RH strain, were obtained by peritoneal washes of infected mice. Activated macrophages were infected with a 10 to 1 tachyzoite macrophage ratio and some were treated with anti-TGF-beta IgY during the 2h interaction period until 24h. NF-kappaB, IkappaB-alpha and IkappaB-beta in noninfected or infected macrophages were visualized in a confocal microscope by immunolabeling. NF-kappaB was localized in the nucleus of noninfected macrophages. However, nuclei of infected macrophage were negative. After anti-TGF-beta treatment NF-kappaB was normally present in the nucleus of infected and noninfected macrophages. IkappaB-alpha, but not IkappaB-beta, was observed in the nucleus of infected macrophages. In conclusion, TGF-beta induced by *T. gondii* infection regulates NF-kappaB nuclear translocation. Furthermore, IkappaB-alpha is involved suggesting a non-classic mechanism for the control of NF-kappaB by *T. gondii* infection. Supported by CNPq and FAPERJ.

## IM29 - Possible involvement of transforming growth factor-beta1 on the deactivation of a chicken macrophage cell line (HD11) infected by *Toxoplasma gondii*

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*Toxoplasma gondii* infects a wide range of warm blood vertebrates, including birds. Chickens might function as a *T. gondii* reservoir because they are highly infected. Thus, we are studying how *T. gondii* behaves in chicken macrophages. Nitric oxide (NO) production is one of the most important microbicidal factors of macrophages. However, we have shown that chicken macrophages infected by *T. gondii* produce less NO. TGF-beta is a macrophage deactivator and Smad proteins are the intracellular mediators of its signaling. Therefore, the presence of Smad proteins in macrophages infected by *T. gondii* indicates the involvement of TGF-beta in this evasion mechanism. To demonstrate the involvement of TGF-beta in this system Smad 2 (normal and phosphorylated) was detected in infected macrophages. For this, HD11 were seeded on coverslips, activated with lipopolysaccharide

and cultured with DMEM-F12 for 24h. Tachyzoites (RH strain) were obtained by peritoneal washes of infected mice. Interactions were performed for 2h in a 10 to 1 *T. gondii* macrophage ratio and cells were further cultured. Supernatants after 24 and 48h were assayed for the presence of NO that was inhibited by 40 percent after infection. Infected HD11 presented increased expression of Smad 2 and phosphorylated Smad 2 was translocated to the nucleus. These results indicate that TGF-beta might be involved in the evasion mechanism of *T. gondii* in chicken macrophages.
Supported: FAPERJ, MCT/CNPq

## IM30 - Oral infection with *T.gondii* specifically elicits CXCL12 production in Peyer patches but not in spleen, as related to TGF$\beta$, in experimentally infected mice

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CXCL12 is a chemokine responsible for leukocyte chemoattraction to sites of infection and is produced by mesenchimal or stromal cells present mainly in endothelial tissues. Presence of CXCL12 may also indicate anti-apoptotic processes, conflicting to the presence of TGF$\beta$, a marker of lymphocyte apoptosis, which inhibits activity of macrophages, resulting in Th2 immune response. These products, TGF$\beta$ and CXCL12, could interact in adaptive immune response, especially in the function of discrete cells populations, such as APCs. There are few studies about functions of these molecules in enteric infections. We study the production of TGF$\beta$ and CXCL12 by a SemiQuantitative RTPCR assay in Peyer patches and spleen of experimentally infected mice. Lymphocytes from Peyer patches and spleen were obtained from control mice or after 30day oral infection with 10 cysts of ME49 strain. Organs were aseptically removed and conserved in TriZOL. RNA obtained after extraction was transcribed to cDNA using MMLV reverse transcriptase and OligodT primers. cDNA were amplified by PCR using specific primers for CXCL12, TGF$\beta$ and $\beta$actin sequences, with resolution of fragments in silverstained PAGE. After digitalization, quantitative data was obtained using artificial optical density using the ImageJ freeware. Results were estimated as percent of $\beta$actin production. In spleen, there is a good positive relationship between CXCL12 and TGF$\beta$ production, absent in normal Peyer patches, with low CXCL12 production. Enteric toxoplasmosis induces a substantial increase in CXCL12 production in Peyer patches, with levels similar to spleen production. We also showed that infection slightly increases expression levels of the two cytokines. Increase of CXCL12 expression could be related to homing effect of APC in the Peyer patches, resulting in immune response or tissue regeneration after enteritis caused by *T.gondii* RH infection, but antigen stimulation must be confirm the exact effect and the cells involved in these processes. Financial support: CNPq, LIMHCFMUSP49 and FAPESP (99/04926-6).

## IM31 - Immunization with *Trypanosoma cruzi trans*-sialidase increases the pathogenesis of experimental chronic

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Chagas' disease is an important health problem in Latin American countries, where it is estimated that about 18 million people are affected. The mechanisms leading to the development of chronic chagasic cardiomyopathy (CChC), the most common symptomatic form of the disease, are unknown. In this work we investigated whether the immunization with *trans*-sialidase (TS), an immunodominant *Trypanosoma cruzi* antigen affects the development of chronic myocarditis in a mouse model of infection. Three weeks after the last dose, mice were challenged with 100 trypomastigotes of *T. cruzi* Colombian strain. Groups of mice were sacrificed in the acute and chronic phase of infection for histologic examination of hearts and blood was collected for detection of anti-TS antibodies by ELISA. Naked DNA immunization with a plasmid containing the catalytic domain of TS alone or in combination with recombinant TS immunization induced the production of high levels of anti-TS antibodies and IFN-gamma production, but did not affect the control of parasitemia during the acute phase of infection with Colombian strain *T. cruzi*. However, mice immunized with TS prior to infection developed a more intense myocarditis 4 months after infection, compared to control mice. At this timepoint of infection, spleen cells from TS-immunized mice produced higher IFN-gamma levels when compared with controls. Although there were no significant differences on myocarditis between immunized and control groups after 7-8 months of infection, mice previously sensitized with TS had significantly more severe cardiac conduction disturbances in electrocardiographical analysis compared to infected controls, such as intraventricular conduction disturbances, atrium-ventricular blocks and extrasystoles. Our results indicate that immune responses against *T. cruzi trans*-sialidase influence or participate in the pathogenesis of chagasic myocarditis.
Financial support: FIOCRUZ, FAPESP.

## IM32 - Comparison of immunological and inflammatory responses after Colombian strain *Trypanosoma cruzi* infection of mice with different genetic backgrounds

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The pathogenesis of chronic chagasic cardiomyopathy (CChC), the most severe form of Chagas' disease, are yet to be disclosed. This disease constitutes an important health problem in Latin America, where about 18 million people are infected by *Trypanosoma cruzi*. The development of CChC may be related to events occurring in the acute phase of infection. Here we compared the immune and pathological responses of isogenic strains of mice infected with Colombian strain *T. cruzi*. Mice of A strain were highly susceptible to *T. cruzi*infection, with high parasitemia and mortality when compared to BALB/c and C57BL/6 mice. In the chronic phase, BALB/c mice developed more intense myocarditis and fibrosis compared to C57BL/6 mice. During the acute phase, a severe inflammatory reaction was detected in the hearts of mice from the 3 strains, although a higher inflammatory response and parasitism was observed in hearts of mice from A strain when compared to the other strains. Mice of A strain also had lower levels of IFN-$\gamma$, IL-4 and IL-10 in cardiac tissue than mice of BALB/c and C57BL/6 strains. In the acute phase of infection, serum titers of anti-*T. cruzi* IgG1 and IgG2a antibodies were significantly higher in mice of BALB/c and A strains compared to mice of C57BL/6 strain. Similar results were obtained regarding *trans*-sialidase specific antibodies. A strain mice had a predominance of IgG2a over IgG1 antibodies, where as BALB/c mice had the opposite. BALB/c mice also had significantly higher levels of *T. cruzi*- specific antibodies than C57BL/6 mice during the chronic phase of infection. The results indicate that anti-*T. cruzi*antibody production does not correlate with resistance to infection. The influence of this humoral immune response, as well as of other immunological parameters on the development of CChC, is being investigated.
Financial support: FIOCRUZ, FAPESP and NIH.

## IM33 - Bone Marrow Transplantation versus Stem Cell Mobilization with G-CSF in a Mouse Model of Chronic Chagasic Cardiomyopathy: A Pilot Study

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Chagas' disease, caused by the *Trypanosoma cruzi* protozoan, is a major health problem in Latin America. Hematopoietic stem cells have come to focus due to their ability to transdifferentiate into different cell types. In experimental Chagas' disease, transplantation of bone marrow cells caused a decrease in fibrosis and inflammation in Chronic Chagasic Cardiomyopathy (CChC). The aim of this study was to compare direct bone marrow cell injection versus peripheral mobilization with G-CSF as treatment for CChC. Female C57BL/6 mice were infected with Colombian strain *T. cruzi* trypomastigotes and parasitemia was evaluated to confirm infection. Eight months after infection, animals were splenectomized and 2 months later divided into three groups as follow: (A) BMT: injected with $4x10^7$ mononuclear BM cells from male C57BL/6 EGFP transgenic mice per intravenous route; (B) G-CSF: treated with G-CSF (filgrastim) 200 $\mu$g/kg/day IP for five days in two cycles with an interval of 7 days; (C) Infected control: injected with saline. Animals were sacrificed 75 days after treatment regimen. Electrocardiogram evaluations were performed before and 30 and 60 days after treatment. All animals had the same severity of cardiac conduction disturbances prior to treatment. Either in BMT and G-CSF group we could observe cardiac electrical activity improvement in 30 and 60 days after therapy. The hearts were fixed and stained with hematoxilin and eosin or Masson's trichrome to determine percentage of inflammation and fibrosis respectively. In this experiment there was a reduction in fibrosis and inflammation in both treated groups. EGFP+ myocytes and vessels were present in BMT group as demonstrated by confocal mycroscopy. Fluorescent in situ hybridization of Y+ chromosome confirmed the presence of these cells in treated hearts with BMT. These data suggest that G-CSF treatment may be an alternative approach to BMT in CChC. More investigation, however, is needed to clarify this issue.
Financial support: IMBT, CNPq, FIOCRUZ and FAPESB

## IM34 - IMMUNOGLOBULIN G AVIDITY IN DIAGNOSIS OF ANIMAL TOXOPLASMOSIS

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*Toxoplasma gondii*, worldwide highly infective protozoan parasite, infects humans and animals, with economic losses by fetal infection and abortion in veterinary medicine, related to acute or recent infections. Diagnostic serology for anti-*T.gondii* IgG does not discriminate between acute and chronic infections. The selection of high affinity B cell clones

during the immune response induces increasing immunoglobulin avidity, which could be predictive factor of infection period. Despite its usefulness, there are few reports on their use in veterinary medicine. In this study, we measure *T.gondii* IgG avidity in experimentally infected cattle, sheep, rabbit, cat and dogs. Groups of at least 4 animals were infected and peripheric blood cells (PBC) were obtained before and during acute and chronic infection. Cyst was detected in 6 weeks or 6 months after infection by B1 gene PCR. Avidity IgG ELISA was calculated by several approaches using both single serum dilution or serial dilutions and titers (AVT), using 6M urea elution. Avidity was effective in predicting infection period in all species, but diverse efficiency was observed in each species or determination method. The speed of avidity maturation is affected by the corporal mass of the infected animals or their susceptibility to the agent. PBC proliferation induced by the antigen corroborate that the avidity is dependent of cell selection. There are no relationship between antibody titers and tissue cysts, as demonstrated by PCR. Cysts were more frequent in recent six weeks infections as compared to chronic animals. In cats, the excretion of oocysts in the feces occurred very early in the infection, with low avidity antibodies. This fact could suggest that the presence of antibodies interferes with the production of oocysts. All those data shows the importance of IgG avidity in veterinary toxoplasmosis, a powerful tool for the temporal diagnosis of this zoonosis.

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## IM35 - Opsonization of amastigotes impair nitric oxide production by activated macrophages in the presence of an adjuvant

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In C57BL/6 mice, L. amazonensis causes a chronic lesion which does not heal, while infection of the same mouse strain with L. major is self-healing. This difference in behavior between the two parasites seems to be caused by a smaller inflammatory response to L. amazonensis. In order to achieve healing of this infection, vaccination with Leishvacinâ (Biobrás, Montes Claros, MG) together with the adjuvant Corynebacterium parvum has been performed. Our studies have revealed that, in spite of a significant decrease in lesion sizes and increase in IFN-g production, vaccination fails to completely protect C57BL/6 mice from infection with L. amazonensis. In fact, the number of parasites per gram of tissue is not different between vaccinated and control mice. When serial dilutions of the whole lesion was performed, the difference of the estimated number of parasites was only of one order of magnitude. Hence, we decided to investigate the effect of L. amazonensis antigens, adjuvant and the incubation of parasites with normal mouse serum in the capacity of IFN-g-activated macrophages to make nitric oxide (NO) in response to L. amazonensis or L. major amastigotes. Activated macrophages respond with lower levels of NO when infected with L. amazonensis amastigotes when compared to L. major. C. parvum and parasite antigens induced high amounts of NO, which were not increased by amastigotes. However, when amastigotes were pre-incubated with normal mouse serum, the production of NO by activated macrophages in the presence of C. parvum was completely abrogated. We hypothesize that, although C. parvum increases the production of IFN-g in vaccinated mice, the capacity of macrophages to produce NO and therefore kill Leishmania may be impaired by factors in serum which bind to amastigotes.

## IM36 - IgG ISOTYPES TO PLASMODIUM VIVAX APICAL MEMBRANE ANTIGEN-1 (PvAMA-1) IN SUBJECTS EXPOSED TO MALARIA IN BRAZIL

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The Apical Membrane Antigen-1 of Plasmodium species (AMA-1) is have been characterized as target for protection and as a possible malaria vaccine due to the restrict polymorphism presented and its role in the erythrocyte invasion. Malaria in the Brazilian Amazon is hypo- to mesoendemic with unstable transmission and the Plasmodium vivax is the prevalent species. Thus, the association of exposure to malaria transmission and IgG isotype to AMA-1 of Plasmodium vivax (PvAMA-1) was examined. For this purpose, we selected two distinct groups of subjects who had been exposed to different malaria transmission in Brazil, reporting one single P. vivax malaria episode (n=59) or more than ten previous episodes by P. vivax and/or P. falciparum (n=117). Recombinant protein which represents the PvAMA-1 was used in ELISA to measure the subclasses Index of Reactivity (IR) and their frequencies. A higher number of sera (89%) from subjects who experienced more than ten malaria episodes was IgG positive as compared to those with a single P.vivax episode (59%). The IgG IR were also significantly higher in individuals constant exposed (IR=3.79±2.38) than individuals who reported one previous malaria (IR=1.54±1.30) (p¡0.001). The IgG1 was the prevalent isotype and its frequency and IR were also significantly higher in individuals straight exposed compared to the short-term exposed individuals. The IgG2, IgG3 and IgG4 frequencies to PvAMA-1 were similar (approximately 30%) for subjects comprising the two groups. However, the IgG3 IR was significantly higher in individuals constant exposed (up to 2.3). Our results suggested a correlation between exposure to malaria and high levels of IgG1 and IgG3 anti-PvAMA-1 in areas of unstable transmission in Brazil

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## IM37 - TLR4- AND IL4-DEPENDENT INDUCTION OF IMMUNE MODULATION AFTER TREATMENT WITH GLYCOINOSITOLPHOSPHOLIPIDS (GIPLs) EXTRACTED FROM *Trypanosoma cruzi*.

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*Trypanosoma cruzi* is the causative agent of Chagas disease. The mechanisms of interaction between the parasite and the host cells are not completely characterized. Molecules present in the surface of the parasite and of the host cells are responsible for signaling and triggering the immune response. Some host receptors for molecules of the *T. cruzi* have been described. Recently, our group has demonstrated that the epimastigote glycoinositolphospholipid (eGIPL), a major glycolipid also present in the surface of trypomastigotes, is dependent on Toll-like receptor 4 (TLR4) expression for NF $\kappa$B activation. To investigate the effects of GIPL in the acquired immunity, we analyzed the levels of anti OVA specific IgE in the serum of Balb/c mice immunized with OVA and treated with GIPL. At day 14 after the first immunization with OVA/alum, we observed a marked increase in the IgE serum levels in mice that received the GIPL injection one day before the second OVA/alum immunization, at day 7. The frequency of eosinophil precursors in the bone marrow of treated and control mice was also evaluated. We found that the GIPL treatment also increases the frequency of eosinophil precursors, in an IL4 dependent way. This phenomena is dependent on TLR4 functional expression, as mice mutated in this receptor (C3H/HeJ) are not affected by the GIPL treatment. We have also analyzed the effects of GIPL in dendritic cell maturation and found that 5 hours after its injection, CD11c spleen cells display higher expression levels of the CD86, but not of the CD80 molecules.

## IM38 - cDNA vaccination from *Lutzomyia longipalpis* salivary proteins protects golden hamster against *Leishmania chagasi*

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The saliva of blood sucking arthropods contains pharmacologically active substances that affect the vertebrate host's hemostatic, inflammatory and immune response. Some reports in the literature have demonstrated that the immunization of mice with proteins or cDNA from sand fly salivary gland protected the animals against a challenge composed of parasite plus saliva. Initially, we developed a visceral leishmaniasis (VL) model in golden hamsters, inoculating them with *Leishmania chagasi* plus saliva from *Lutzomyia longipalpis* in the ear dermis. We observed that hamsters developed VL two months post-infection and most of the animals died in six months post-infection. Next, we used different cDNAs from salivary proteins from *L. longipalpis* as well as sonicate of the salivary glands to immunize hamsters. They were immunized three times with 15 days of interval. Fifteen days after last immunization, the animals were injected in the ear dermis with *L. chagasi* ($10^5$) plus saliva (0.5 pair) from *L. longipalpis*. Our study showed that at least two cDNA (LLsp44, and LLsp11), which induce delayed-type hipersensitivity (DTH) responses after immunization, and SGH were able to protect against *L. chagasi* at least two months after challenge. Although, the injection of LLsp45 and Llsp61 induced a strong humoral response against salivary proteins, they did not protect the animals against *L. chagasi*. Taken together, our results showed that a cDNA vaccine protected animals from a subsequent challenge of parasite plus saliva. Interestingly, the mechanism responsible for such protection seems to involve a DTH reaction, since the cDNAs that induced only antibody production did not show any protective effect. Therefore, our results confirm previous data showing that it is possible to immunize against *Leishmania* using as target products from salivary glands from vectors responsible for the parasite transmission. Suppoted by CNPq and NIH.

## IM39 - Fas-L MOLECULE REGULATES CARDIAC INFLAMMATION IN *Trypanosoma cruzi* INFECTION

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Chagas' disease is caused by the protozoan *Trypanosoma cruzi* and has a widespread distribution in Americas. In the acute and chronic phases of the infection, it is observed cardiac cellular inflammatory infiltration, tissue parasitism, fibrosis and cardiomyocytes death. However, it is not clear what cytotoxic cellular populations and lytic molecules are involved. In this work we studied the importance of Fas-L, one of the major cytotoxic pathways, in the cardiac inflammatory response induced by the infection. We used BALB/c and Fas-L deficient mice *(gld/gld)* infected with *Trypanosoma cruzi* Y strain and observed similar parasitemia in both lineages, but higher mortality rates in *gld/gld*. Interestingly, on $15^{th}$ day post infection we observed cardiac parasite nests in both mice groups but significantly reduced cellular inflammatory infiltration in *gld/gld*, when compared to BALB/c mice. Accordingly, tissue damage was higher in BALB/c infected mice, as observed by CK-MB activity. Flow cytometry-based cardiac cells analysis showed higher numbers of $CD8^+$ T lym-

phocytes and MAC-$1^+$ cells in BALB/c mice, but similar low levels of $B220^+$, $\gamma/\delta$ T cells and $CD4^+$ T cells in both groups. Besides, we found a Th1 cardiac response in BALB/c, with high levels of IFN-$\gamma$ and IL-2, but a mixed Th1/Th2 response in *gld/gld*, with also high levels of IL-4 and IL-10. We observed a predominant cardiac T cell population with phenotype $CD3^+/CD8^-/CD4^-$ in both BALB/c and *gld/gld*. However, the expression of many adhesion molecules and T cells markers were down regulated in the *gld/gld* group, such as ICAN, NCAN, VCAN, CD2, LFA-1 and CD69. We conclude that Fas-L is important in the regulation of myocarditis induced by *T. cruzi* , far beyond its known cytotoxic function.

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## IM40 - Effect of hypoxia on macrophage infection by *Leishmania amazonensis*

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Pathological conditions such as cutaneous and mucocutaneous leishmaniasis can cause the formation of area with low oxygen in tissues. Experimental hypoxia has been reported to induce phenotypic adaptations in macrophages. In this study we compared the effect of a 5% oxygen tension (hypoxia) with a normal tension of the 21% oxygen (normoxia) on macrophage infection by the protozoan parasite *Leishmania amazonensis*. Macrophages from different sources (human primary macrophages derived from peripheral blood monocytes, human tumor cell line U937, murine tumor cell line J774 and peritoneal murine macrophages) were exposed to hypoxia or normoxia during 1 hour or 24 hours. Murine macrophages and human U937 cells exposed to hypoxia showed a reduction of the percentage of infected cells and of the number of intracellular amastigotes per cell. Observation of the kinetics of infection indicated that hypoxia did not depress *L. amazonensis* phagocytosis but induced macrophages to reduce intracellular parasitism. Furthermore, hypoxia did not act synergistically with gamma interferon and bacterial lipopolisaccharides in macrophages to induce killing of parasites. In contrast, human primary macrophages derived from peripheral blood monocytes exposed to similar conditions did not show a significant reduction in the percentage of parasitized cells. Experiments also indicate no correlation between nitric oxide production and control of infection in macrophages under hypoxic condition. Thus we provide the first evidence that hypoxia, which occurs in various pathological conditions, can alter macrophage susceptibility to a parasite infection. Supported by FAPESP, CAPES and CNPq.

## IM41 - Isotypes Patterns Of Immunoglobulins: Indicators Of The Clinical And Parasitism Status In Brazilian Dogs With Naturaly Infected By *Leishmania chagasi*

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Dogs are considered the most important vertebrate reservoir of the disease due to its greater prevalence and the frequency of *Leishmania* amastigotes in the skin of these animals. In CVL, the immunological mechanism underlying the susceptibility or resistance to severe disease remains for less defined. Polyclonal activation of B-cells leading to high titers of circulating antibodies are find of the course of L. chagasi infection and the detection of IgG anti-Leishmania antigens is an important diagnostic to in identifying case of CVL. In the present study were evaluated 40 naturally infected dogs by *Leishmania chagasi*with different clinical features and 20 non-infected dogs as a control group. The infected animals were classified into two categories: according to their clinical symptoms and intensity parasitism tissues. These animals were submitted for a detail analysis of serological parameters by ELISA using a specific monoclonal anti-canine isotype antibodies (IgA, IgM, IgE, IgG, IgG1 and IgG2) employing a soluble L. chagasi antigen. The result shows an association between high levels of IgG1 with asymptomatic animals as well as in the low parasitism group. In the other hand, higher levels of IgG, IgG2, IgA, IgM and IgE was observed in oligo, symptomatic and high parasitism groups. Our results emphasize that progression of disease in dogs is characterized by appearance of specific isotypes of immunoglobulins (mainly IgA, and IgE) with may contribute to aggravation of clinical status of the infection dogs. Furthermore, the elevated production of IgA and mainly IgE in oligo, symptomatic and in the high parasitism group, might suggest an associated of this clinical feature and the parasite intensity with a type 2 immune response.

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### IM42 - Study of serological reactivity by western blotting of dogs with visceral leishmaniasis against antigens of *Leishmania chagasi*and *Leishmania braziliensis*

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Dogs are an important reservoir of American Visceral Leishmaniasis. Approaches towards developing vaccines and therapeutic options to treat dogs with canine visceral leishmaniasis (CVL) are an intense area of research. Our studies are of importance towards the establishment of parameters to evaluate the effectiveness of vaccines and new drugs against CVL. The aim of present work is to identify the major antigens recognized by antibodies of dogs with CVL (symptomatic and asymptomatic) and vaccinated animals. Soluble antigens of *L. chagasi*, *L. braziliensis*and the saliva of *Lutzomyia longipalpis*were employed. The *L. braziliensis*and the saliva antigens are part of the composition of an anti-CVL vaccine. Western blot experiments were conducted by separating the antigens by SDS-PAGE followed by electro-transference to a nitrocellulose membrane, incubation with dog antiserum followed by detection with anti-dog IgG/alkalyne phosphatase and visualization with the substrate NBT-BCIIP. We have identified 17 major bands in *L. chagasi*, 18 major bands in *L. braziliensis*extracts and four protein bands present in the saliva of *Lu. longipalpis*. These preliminary results will aid in the identification of the specific constitution of the vaccines under development. The immunogenicity of different antigens will also contribute towards future strategies for the development of recombinant vaccines, which will be our next direction of research.
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### IM43 - An Alternative Immunohistochemical Method to Detect Leishmania Amastigotes in Paraffined Tissues of Cutaneous and Visceral Human Leishmaniasis Lesions

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Several methods are currently employed for diagnosis of CVL, including microscopic detection of the parasite in bone marrow and lymph node aspirates, demonstration of specific antibodies anti-Leishmania in sera from infected animals, isolation of the parasite by in vitro culture or by laboratory animal inoculation. However, a definitive diagnosis is based on the actual detection of the parasite, which is conventionally achieved by examining Giemsa stained smears or histopathological sections stained by Hematoxilin and Eosin (HE). These methods have a low sensitivity and therefore they are often inconclusive. This is particularly true in human skin tissues in cutaneous leishmaniasis. In previous study (Tafuri et al., 2004) described an alternative immunohistochemistry technique for immunohistochemical detection of leishmanial amastigotes in canine tissues. In this work we test this new immunohistochemistry protocol to detect amastigotes forms of Leishmania in Tegumentar and Visceral Human Leishmaniasis lesions. Fragments of skin and livers (biopsies) were gently provided by Santa Casa Hospital and Tafuri Anatomical Pathology Laboratory, Belo Horizonte, MG. All the tissues were previously prepared for histopathological study (HE) in order to define the presence of intracellular amastigotes forms of Leishmania. Fragments of skin and liver with intense parasitism were used for the immunocytochemistry test. In all fragments tissue immunolabeled amastigotes forms of Leishmania were easily observed within macrophages in all selected tissues (skin and liver biopsies) using the streptoavidin-biotin immunohistochemical method. The goal of this method is a canine hyper immune serum employed as primary antibody. In addition, the second antibody used was not specific to canine immunoglobulin characterizing a cross immune reaction. The immunoperoxidase protocol employed in this study, which is based on the use of serum from naturally infected dogs, is inexpensive and readily available when compared to monoclonal or polyclonal anti-Leishmania antibodies (Bourdoiseau et al., 1997; Livini et al., 1983). Although the secondary antibody (LSAB+ Kit, Dako) is not specific to the dog serum, it resulted in cross immunoreaction, and this method proved to be as specific as the use of monoclonal or polyclonal anti-Leishmania antibodies. Our results indicate that this technique could be an useful tool for human leishmaniasis histopathological studies.
Financial Support: FAPEMIG, CNPq, UFMG

### IM44 - Phenotypic profile of splenocytes during acute *Trypanosoma cruzi* infection in dogs

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The principal objectives of studies on immunity to *Trypanosoma cruzi*are the understanding of immunological mechanisms involved in resistance as well as the pathogen-

esis of Chagas disease. Several studies have defined CD8 T lymphocytes, IFN-y and macrophages as important elements involved during the acute phase of infection in the control of parasite replication. Previous studies in our laboratory have demonstrated that blood (BT) or metacyclic (MT) trypomastigotes induce differentiated immunological responses, which may affect the development of Chagas disease. Phenotypic analysis show that all infected dogs have a progressively increase in THY-1, CD8 T cell and a decreased in CD21 and CD5 cells. The levels of circulating monocyte CD14 and CD4 T lymphocytes are maintained. However, dogs infected with MT showed a high levels of CD8 T cells in comparison to BT in peripheral blood. In the present study was available the spleen compartment in experimental Chagas disease in dogs. Our date showed a decrease in the percentage of CD4 T cell and an increase in CD8 T cells. Despite higher levels of CD8 splenocytes detected in all infected dogs, a more drastic increase of this population was observed in the spleen of dogs infected with MT. However, in dogs infected with BT, higher levels of CD21 B cells was observed in comparison to the control group as well as to dogs infected with MT. Taken together, our data clearly demonstrated the importance of inoculum on the induction of differentiated immunological responses, more intense in dogs infected with MT. Moreover, our findings suggested that CD8 T cells may play an important role during acute Chagas disease in dogs, inducing pathology and CD21+ cells inducing protection.

## IM45 - Effectiveness of Posaconazole in the treatment of the acute phase of Chagas disease in IFN-$\gamma$ deficient mice

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Benznidazole (BZ) is a drug used in the treatment of the Chagas disease, that presents a limited effectiveness and considerable side effects. Interferon-$\gamma$ (IFN-$\gamma$) is described as an important mediator of the activity of BZ. Posaconazole (POS), an inhibitor of sterol biosynthesis, has been successfully used in the treatment of experimental Chagas disease. In this work, we test the effectiveness of POS to cure IFN-$\gamma$deficient mice, infected with *Trypanosoma cruzi* and treated in the acute phase. C57Bl/6 mice, IFN-$\gamma$ producers, and knockout mice (KO), deficient in the production, were divided in groups of 15 animals, infected with *T. cruzi* and treated in the acute phase with POS and BZ. The activity of the drugs was determined by the parasitaemia and mortality of the mice, observed up to 60 days post infection. After this period, mice with parasitaemia not-detectable to the microscope, were submitted to the hemoculture to define cure. Normal infected mice treated with both drugs presented cure and survival of 100%. The IFN-$\gamma$KO infected mice treated with POS presented cure of 15% and survival of 45%, and those treated with BZ presented cure and survival of 0%. IFN-$\gamma$KO infected mice treated with BZ, had the parasitaemia reactivated two days after ending the treatment, with the occurrence of a parasitemic peak, followed by animals death. The same mice treated with POS presented a reactivation of the parasitaemia 9 days after ending the treatment with low parasitaemia and no death. Infected and non treated KO and normal mice presented high parasitaemia and mortality of 100% on the 15th and 30th days of infection, respectively. The results show how essential IFN-$\gamma$ is for the effectiveness of the treatment with both drugs. In the absence or deficiency of IFN-$\gamma$, BZ was completely ineffective while POS still presented some efficacy. Supported by:CAPES/CPqRR-FIOCRUZ

## IM46 - CD4+ T Lymphocytes are essential for the effectiveness for the treatment with Benznidazole in the acute phase of murine Chagas disease

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Benznidazole (BZ) is used in the chemotherapy of Chagas disease, with an efficacy of 76% in the acute phase and only 8% in the chronic phase. With an increase in the cases of HIV infected individuals and people under immunosuppressive therapy after organ transplants, the reactivation of chronic infections has been observed, followed by meningoencephalitis and patient's death, can be explained by a decrease in the type 1 immune response that has CD4+ T lymphocytes as one of the main cells. The present study aims to assess the efficacy of BZ in the cure of *Trypanosoma cruzi* infected knockout mice (KO) for the type II Major Histocompatibility Complex (MHC), therefore deficient in the production of CD4+ T lymphocytes. C57Bl/6 mice (n=16), normal producers of this lymphocytes and KO (n=16), deficient in the production, were infected with T. cruzi and treated in the acute phase, with BZ. Animals infected and not treated were used as control (n=10). The parasitaemia and mortality were observed up to 60 days after infection. After this period, mice with negative parasitaemia were submitted to hemoculture, to verify the cure. Normal infected mice presented 100% of cure and survival, while KO infected mice presented 0% cure and 6% survival, with reactivation of the infection seven days after the end of the treatment. The parasitaemia was low and persistent enough to kill 15 out of the 16 infected animals (94%). Normal and KO mice, which were not treated, presented high parasitaemia and mortality of 100% on the 15th and 30th days after infection, respectively. The results show how essential CD4+ T lymphocytes is for the effectiveness of the specific treatment of murine Chagas disease, suggesting that in immunodeficient and/or immunosuppressed patients the treatment of the BZ reactivation of Chagas disease may have its efficacy reduced. Financial support: CAPES/CPqRR-FIOCRUZ

## IM47 - Analysis of the humoral response and protection induced in C57Bl/6j mice immunized with irradiated *Toxoplasma gondii* tachyzoites challenged with VEG strain

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Toxoplasmosis, usually benign protozoan disease, leads to ocular lesions in normal individuals or severe disease in immunocompromised ones, with brain lesions and death. Fetal infection is also severe and debilitating and occurs in the acute infection of pregnant women. The same diseases could occur in farm animals, with abortion and economic losses. The available vaccine for sheep does not induce complete protection, similar to several experimental protocols, using thermo sensitive strains or recombinant proteins. Radiation sterilized tachyzoites (RST) could be an alternative, as when used for immunization in mice, they induced expressive decrease of cysts formation in Type II virulent strains challenge and a partial protection against type I lethal challenge, with immunity similar to chronic infection. There are few strains of *T.gondii* circulating in the world and a vaccine candidate must protect against the main 03 infecting strains. To test this fact, C57Bl/6j mice were immunized with three doses of RST biweekly by intraperitoneal route (i.p.). After two weeks, these mice were challenged with 10 cysts of type III *T.gondii* (VEG) by oral route. Tail blood was collected weekly in standardized filter papers and stored at in freezer. ELISA detected the presence and avidity of IgG antibodies. There were no deaths both in immunized and control mice groups, with gradual increase in IgG antibodies level after immunization with 3 doses of RST. After cyst challenge, there was a significant increase of the antibodies levels and in the avidity maturation of these antibodies. After three months of challenge, all mice were killed, with counts of total brain cysts. The immunized mice presented reduced brain cysts counts when compared with infected mice, showing that RST produced with type I RH strain parasites also promotes the immunity against type III VEG cysts, but more efforts are need towards a sterilizing vaccine. This work was supported by LIMHCFMUSP49, CAPES and CNPq.

## IM48 - Canine Visceral Leishmaniasis: evaluation of the chronic inflammatory reaction and parasitism load in skin tissues of dogs naturally infected with Leishmania (Leishmania) chagasi

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Canine visceral leishmaniasis (CVL) is a severe systemic disease caused by Leishmania (Leishmania) chagasi. In Latin America dogs are considered the principal domestic reservoir host to the human disease. CVL is mainly characterized by a high frequency of skin lesions. The histopathological picture of these lesions is presented by a chronic inflammatory reaction associated to a variable parasitism tissue load. The aim this work is to evaluate a number of inflammatory cells in ear, nose and abdominal fragments skin of asymptomatic, oligosymptomatic and symptomatic mongrel dogs. These data was correlated to the parasitism tissue load. Animals from Belo Horizonte, MG and João Pessoa, PB, Brazil, were sacrificed with lethal dose of Sodic Thiopental (33 percent). Skin fragments of ear, nose e abdomen were collected after necropsy, fixed in buffer formalin. The skin tissue sections were dehydrated, cleared, embedded in paraffin for histopathological (HE) and immunohistochemical analysis. Skin cut sections stained by HE were used to quantify the number of inflammatory cells. It was accessed in a morphometrical analysis in a Zeiss Imaging Processing Software (KS300) using sequential steps in a KS300 macro as describe by Maltos et. al (2004). The streptoavidin-peroxidase immunohistochemistry method was carried out for amastigotes detection in all skin paraffined tissue. Immunolabeled amastigotes were quantified by morphometrical analysis using the KS300 software. Our results have been demonstrated higher numbers of inflammatory cells in ear skin tissue fragments. In general, asymptomatic dogs showed similar number of inflammatory cells than control animals. Symptomatic dogs group showed higher numbers of inflammatory cells in all skin tissue fragments (statistical significance in ear and nose). However, the parasitism in all skin fragments tissues of all clinical defined animals was not correlated to the cellular exudate quantification.
Supported by FAPEMIG, CNPq, UFMG

## IM49 - Parotid, mandibular and cervical lymph nodes of dogs naturally infected with Leishmania (Leishmania) chagasi: a histopathoilogical and immunocytochemistry study and its correlation with head skin lesions

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Lymphadenomegaly and skin abnormalities are common clinical signs findings of Canine Visceral Leishmaniases (CVL). The aim of this study was to evaluate the histopathology, tissue parasitism load of lymph nodes (cervical, mandibular and parotid) and skins tissue sections of the external nose and external ear. Twenty-eight mongrel dogs were obtained from the City Hall Zoonosis Department of Belo Horizonte, MG, Brazil. Twenty-two naturally infected dogs with serological positive exams to Leishmania (IFAT - Titles up 1:40; and ELISA) were classified in two different groups: dogs with

lesion on the head (external nose and external ear) and dogs without skin lesions on the head. Six dogs with negative serological exams composed the control group. All animals were killed with a lethal dose (1mL/kg i.v.) of 33 percent Thiopental solution. Fragments of lymph nodes and skins were fixed formalin and processed for histopathological analyses and immunocytochemistry (ICQ) (Tafuri et al., 2004) to detect amastigote forms of Leishmania in paraffin tissue sections. Moreover, all lymph nodes were obtained to prepare tissue touch preparations (smears) for LDU analyses. Animals with head and external ear skin lesions showed higher parasitism numbers in parotid lymph nodes than compared to animals without skin lesions (p equal 0.048, p equal 0.019; respectively. There was no difference between the number of tissue parasites load (LDU and ICQ) of all lymph nodes considering all animal groups (Kruskal-Wallis test). On the other side, parasitism data of parotid lymph nodes showed concordance to parasitism data of skin of the external ear and external nose (p equal 0.019, p equal 0.005, respectively. Parasitism data of mandibular lymph nodes showed concordance to parasitism data of skin of the external nose (p equal 0.021) and parasitism data of cervical lymph nodes showed concordance to parasitism data of skin of the ear (p equal 0.012) (Spearman test ).

## IM50 - EFFECTS OF GLUTATHIONE MODULATING AGENTS (NAC OR DEM) ON LEISHMANIA INFECTIVITY AND NITRIC OXIDE PRODUCTION BY HUMAN AND MURINE MACROPHAGES

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Introduction and Aims: *Leishmania* are obligate intracellular protozoan parasites that infect host macrophages. It is well documented that the control of the infection requires the induction of an immune response capable of activating macrophages to a microbicidal state, which depends mainly on the production of free radicals for killing of the parasites living within macrophages. Glutathione (GSH) is the major intracellular redox buffer and plays a role in protecting cells against oxidant damage, as well as modulates the expression of several genes. We have recently observed that the host response to *L. major* infection can be significantly improved by increasing *in vivo* glutathione (GSH) levels in murine model. We are interested in how GSH modulation could be employed to improve immune responses *in vivo*. For this purpose we evaluated the human and murine macrophage response to *Leishmania* infection and LPS stimulation in the presence of two glutathione modulating agents: N-acetyl-L-cystein (NAC), a GSH precursor, and diethyl-maleate (DEM), a GSH depleting agent. Methods and Results: For determination of macrophage infection by *Leishmania* promastigotes, parasites were stained with CFSE (carboxyfluorescein diacetate, succinimidyl ester) and analyzed using flow cytometry. The nitric oxide production and iNOS expression were determined using the Griess reaction and confocal microscopy, respectively. The effects of GSH modulation on macrophage-parasite interaction through CD11b were also analyzed by flow cytometry. Reducing intracellular GSH levels in human macrophages led to an increased frequency of infected macrophages, correlated with a reduced expression of nitric oxide synthase. In the murine model, macrophage functions can also be improved or impaired by GSH modulation. Conclusions: GSH modulation effects macrophage function and susceptibility to infection by *Leishmania in vitro* and might be a useful pathway to improve the host response against *Leishmania* infection. Financial Support: WHO/TDR, PRONEX/CNPq. e-mails: kjgollob@icb.ufmg.br and privanna@yahoo.com.br

## IM51 - Influence of parasitism in dermal inflammatory focus in canine visceral leishmaniasis by *Leishmania*(Leishmania)*chagasi*

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Introduction and Objectives: The skin is considered an important compartment reservoir of parasites in healthy and sick *Leishmania*-infected dogs. Little is know about the process and mechanisms involved in cutaneous tissue immunopathology in canine visceral leishmaniasis (CVL) presenting different clinical patterns. Methods and Results: In this context, the aim of the present study was to make immunopathological study in ear skin biopsies from asymptomatic (AD, n=12), oligosymptomatic (OD, n=12) and symptomatic (SD, n=15) CVL-bearing dogs as well as 11 healthy controls (NID). These results of microscopical analysis weren't make association statistical analysis between chronic inflammation skin and presence and density of *Leishmania* amastigotes by immunohistochemical approach. In this context, we observed a tendency to increase parasitism and inflammatory patterns according clinical evolution. In the context, great collagen destruction and reticular fibers neoformation was observed. In addition, we evaluated the correlation between histopathological picture of skin lesions/parasitism and leukocytes in peripheral blood (CD4+, CD8+, CD5+, Thy-1+, CD14+, CD21+) accessing by flow cytometry. Statistical analysis showed a positive correlation on AD between skin parasitism both phenotypes T CD4+ (r=0,57) and B CD21+ (r=075). Remarks and conclusions: In the present study we demonstrated the impact of the intensity skin parasitism in dermal inflammatory focus. The mononuclear cells (monocytes, B-lymphocytes and T-cells) observed in the inflammatory focus aren't capable to de-

stroyed all the amastigotes of *L. chagasi*. The next target of this work is investigating the phenotype population and subpopulation cells and the role of cytokines on skin compartment to understand the immunopathogical events in canine visceral leishmaniasis.
Support: CNPq, CAPES, FAPEMIG, UFMG, FIOCRUZ& UFOP e-mail: giunchetti@nupeb.ufop.br

## IM52 - IMMUNOHISTOCHEMICAL AND MORPHOMETRICAL STUDY OF COMPLEMENT RECEPTOR TYPE 3 (CR3-CD11b/CD18) AND COMPLEMENT RECEPTOR TYPE 4 (CR4-CD11c/CD18) IN LIVER OF DOGS NATURALLY INFECTED WITH Leishmania (Leishmania) chagasi

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Canine Visceral Leishmaniasis (CVL) is a zoonosis and a chronic disease caused by a protozoan of the genus Leishmania. In the New World the causative agent is Leishmania (Leishmania) chagasi. The dog is the domestic reservoir for human visceral leishmaniasis and many of the clinical and pathological signs observed in these animals are similar to the pattern of the disease in human beings Previous works have demonstrated that complement receptor type 3 (CR3) and complement receptor type 4 (CR4) are responsible for the successful phagocytosis and survival of the parasite inside macrophages. Immunohistochemical and morphometrical analysis of the CR3 and CR4 expression were carried out to characterize and quantify the receptors livers cells tissue expression, respectively. In this study we have characterized and determine the number of cells target with CD11b, CD11c and CD18 complement proteins. Thirty naturally infected animals from Zoonosis Department of Belo Horizonte City Hall, positive serological exams to Leishmania (IFAT and ELISA), were divided in groups: asymptomatic, oligosymptomatic and symptomatic (weakness, cutaneous lesions, alopecia, and clinical anemia) animals. The dogs were sacrificed with lethal dose of Thionembutal 33 percent (1,0mL/Kg). During necropsy, small samples of liver were collected and fixed in freezing tissue medium and cut in cryostat. The immunocytochemistry technique (streptoavidin-peroxidase method) was carried out to determine the number of cells target with CD11b, CD11c and CD18 complement proteins. The number of cells target in twenty microscope fields was determinate with a 40x objective of an Axiolab light microscope (Zeiss). There was no statistical differences considering the defined clinical animals (asymptomatic, oligosymptomatic and symptomatic) (p¿0,05). However, infected animals showed higher number of cells marked than the controls (p¡0,05). Furthermore our results will be associated with the hepatic parasitism load and the number of macrophages target with MAC387 antibody. Supported by: CNPq, FAPEMIG, UFMG

## IM53 - HISTOPATHOLOGICAL STUDY OF HEPATIC GRANULOMAS IN CANINE VISCERAL LEISHMANIASIS

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Canine Visceral Leishmaniasis is a zoonosis and a chronic disease caused by a protozoan of the genus Leishmania. In the New World the causative agent is Leishmania (Leishmania) chagasi. The dog is the domestic reservoir for human visceral leishmaniasis and many of the clinical and pathological signs observed in these animals are similar to the pattern of the disease in human beings. In this study we have quantified and measure the tissue granulomas considering defined clinical animals. Seventy-five infected animals with positive serological exams to Leishmania (IFAT and ELISA) were divided in three clinical groups: asymptomatic, oligosymptomatic and symptomatic (weakness, cutaneous lesions, alopecia, and clinical anemia) animals. The dogs were sacrificed with lethal dose of Thionembutal 33 percent (1,0mL/Kg). Liver fragments were collected and processed for histopathological and immunohistochemical studies (HE). The number of granulomas was determined considering twenty microscope optic fields using a 40x objective of an Axiolab light microscope (Zeiss). The images viewed on a computer video screen were obtained by means of the software and relayed to a computer-assisted image analysis system. (Kontron Electronic/Carl Zeiss, Germany). Using a digital pad the total diameter of granulomas was measured and the results were expressed in squared micrometer. The streptoavidin-peroxidase immunohistochemistry method was carried out for amastigotes detection in all skin paraffined tissue. The number of granulomas was higher in the asymptomatic group and it was statistical different as compared to the symptomatic dogs (p lower than 0,05). The diameter of granulomas was not different between three clinical groups (p higher than 0,05). In addition, there was significant correlation between the number and diameter of granulomas (p=0,016) and between the diameter of granulomas and tissue parasitism (p=0,039). We concluded that asymptomatic dogs could have shown higher immunological cellular response than the others infected dogs. Supported by: CNPq, FAPEMIG, UFMG

## IM54 - ANALYSIS OF LYMPHOCYTE POPULATIONS BEARING NK CELL MARKERS IN THE LIVER OF Trypanosoma cruzi ACUTELY-INFECTED MICE: INCREASE OF PanNK+ NK1.1- TCR gamma delta T CELLS.

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Infection of mice with Trypanosoma cruzi (Y strain) results in an acute phase with parasitaemia. Despite this, we have not being able to find T. cruzi nests in the livers, although an inflammatory reaction is present at this organ. In this work we characterized the leukocytes infiltrating the liver parenchyma during the acute phase of the infection. Natural killer cells increased 3-8 fold times early after infection (day 8), while T and B lymphocytes increased at a later point (day 14). The NK nature of the expanded population was confirmed in C57BL/6 mice, most PanNK+CD3- cells co-expressing the NK1.1 marker. Among CD3+ lymphocytes, those most notably increased were the CD4-CD8+ and CD4-CD8- ones. A significant fraction of these lymphocytes, as well as CD4+ lymphocytes, were positive for the PanNK marker. The possibility these PanNK+ T lymphocytes were classical NKT cells was discarded considering that in the C57BL/6 strain most these cells did not express the NK1.1 marker and also because they did not show a preferential usage of Vbeta8 in their TCRs. CD8+PanNK+ cells in the liver of T. cruzi infected mice were mostly TCRalphabeta positive, but those CD4-CD8- PanNK+ T cells expressed the TCRgammadelta receptor. COnclussions: NK cells and T cells expressing the PanNK marker, but not NKT cells, are notably increased in the livers of T. cruzi acutely-infected mice. Among PanNK-expressing T cells, the most important increases occur in CD8+TCRalphabeta and CD4-CD8-TCRgammadelta cells. supported by FAPESP and CNPq

## IM55 - Canine visceral leishmaniasis: relationship of the immunopathologic aspects on spleen compartment.

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Introduction and Objectives: Canine visceral leishmaniasis (CVL) is a disease caused by the protozoan Leishmania chagasi and transmitted by the bite of phlebotomine sand fly vectors. The main clinical findings are skin lesions, hepatosplenomegaly, lymphadenopathy, glomerulopathy and anemia. Studies on histophatology in CVL present only report microscopic lesions. Methods and Results: In this context, the aim of the present study was to make immunopathological study in spleen biopsies from asymptomatic (AD, n=12), oligosymptomatic (OD, n=12) and symptomatic (SD, n=16) CVL-bearing dogs as well as 11 healthy controls (NID). These results of microscopical analysis of the spleen were make association statistical analysis with different histopathological results and parasitism level by immunohistochemical. In addition, we evaluated the correlation between histopathological picture of spleen lesions/parasitism and leukocytes in peripheral blood and splenocytes (CD4+, CD8+, CD5+, Thy-1+, CD14+, CD21+) accessing by flow cytometry. Our data demonstrated that frequency in all infected dogs presented histopathological pattern consistent with white pulp depletion with macrophage infiltrated, red pulp hypertrophy/hyperplasia and congestion, in comparison to NID. Statistical analysis suggested that cell reactivity in different compartment in spleen may be stimulated by parasite load. The correlation was presented among peripheral blood and histological parameters by AD in CD4+, CD14+ and CD21+. Taken together, the correlation was presented between splenocytes and histological parameters by AD in CD4+, CD21+ and OD in CD21+. Remarks and Conclusions: These findings suggest participative reaction in leukocytes emigration from peripheral blood to distinct compartment in spleen reporting of the mononuclear cells regarding immune pathological events associated with CVL outcome. Supported by: CNPq, CAPES, FAPEMIG, UFMG, FIOCRUZ& UFOP
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## IM56 - The role of CD8+ T-cells and monocytes in the control of splenic parasitism in dogs naturally infected by *Leishmania chagasi*.

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Canine Visceral Leishmaniasis is an important veterinary problem as well as of public health where this disease is endemic. Dogs are the domestic reservoirs of zoonotic visceral leishmaniasis and good models for the investigation of pathogenicity and immunity related to human visceral leishmaniasis. In this study, groups of naturally infected dogs were classified according to the intensity of spleen parasitism. Forty dogs were used and subdivided into three

groups. Twenty non-infected dogs were used as control group. They were serologically and parasitologically negative for *L. chagasi*. The criteria for infection level used were the LDU ("Leishman Donovan Units"), that is the number of amastigotes per 1000 nucleated cells. The first group, named Low Parasitism (LP, n=13), had LDU of 0-10; the Medium Parasitism (MP, n=16) DU of 11-250 and the High Parasitism group (HP, n=11), LDU of ¿250. In all groups hemogram alterations were analyzed (Global Leukocytes/mm3, Neutrofils/mm3, Eosinofils/mm3, Lymphocytes/mm3 and Monocites/mm3). Cell phenotypes were also evaluated either by analyses of percent or absolute cell values (THY-1, CD5, CD4, CD8, CD21 and CD14) in blood or spleen lymphocytes. Dogs with HP showed low levels of eosinophils and monocytes. Dogs with LP and MP showed an increase of circulating T lymphocytes, but dogs with HP showed lower values for these cell populations. The immunophenotypic study showed the LD groups presented high % of T-cell (Ty1+) mainly TCD8+ subpopulation. Also we observed a decrease in the CD14 cells in the HD groups. These results demonstrate the importance of TCD8+ lymphocyte populations in maintaining/establishing the parasite/host interactions in the animals of low parasite density. The reduction of monocytes (CD14+) in dogs with HP suggests that this population may be recruited to secondary lymphoid organs and may function as antigen presenting cells.
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## IM57 - MICE EXPRESSING NON-FUNCTIONAL TOLL-LIKE RECEPTOR 4 (TLR4) ARE MORE SUSCEPTIBLE TO INFECTION WITH *Trypanosoma cruzi.*

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Toll-like receptors (TLRs) are important components of the innate immune system. TLRs sense pathogen-associated molecular patterns (PAMPs) derived from a variety of pathogens and have been implicated in resistance to infection diseases. At the present moment, there are more than 10 members of the TLR family in mammals. Recently, it has been shown that Myeloid Differentiation Factor 88 (MyD88) KO mice are more susceptible to infection with *Trypanosoma cruzi* (Campos, M. A. et al, *J. Immunol. 172*:1711, 2004). The same study has also shown that TLR2 KO mice did not differ from control mice on mortality, therefore indicating that other TLR(s) and/or receptors that depend on the MyD88 adapter molecule is/are involved in host innate immune response to *T. cruzi*. In the present work, we investigated the impact of functional TLR4 expression on parasitemia and mortality during the infection with *T. cruzi*. We found that TLR4 mutant mice (C3H/HeJ strain) are more susceptible to infection, presenting higher parasitemia and earlier mortality, when compared with C3H/HeN strain. We also analyzed reactive nitrogen intermediates (RNI), IFN$\gamma$ and TNF$\alpha$ production in serum as in spleen cultures, from both mice strains infected with *T. cruzi*. Moreover, *T. cruzi* replication is higher in cultures of peritoneal macrophages derived from C3H/HeJ mice, compared to parasite growth in C3H/HeN macrophages *in vitro*. In conclusion, these results indicate a role for TLR4 signaling in the control of *T. cruzi* infection *in vivo* and *in vitro*.
Financial Support: FAPERJ, CNPq, CAPES.

## IM58 - CROSS-REACTION OF CHRONIC CHAGASIC PATIENTS SERA WITH *Trypanosoma rangeli* AND *T. cruzi* EPIMASTIGOTES AND TRYPOMASTIGOTES.

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Comparative studies of the antigenic composition of *Trypanosoma cruzi* and *T. rangeli* culture epimastigotes share almost 60 % of their soluble antigens. Thus, the geographical overlap of these parasites along with the antigenic similarity is of major importance for the serological diagnosis of Chagas disease. Previous studies using sera from *T. cruzi* or *T. rangeli* infected mice against culture trypomastigotes of these parasites in immunofluorescence assays (IFA) demonstrated a weak serological cross-reactivity. Further Western blot assays using these same serum revelead distinct antigenic profiles for both epimastigotes and trypomastigotes forms of each parasite species. In the present study, the cross-reactivity of chronic chagasic patients serum (cardiac, digestive and indeterminate forms) against culture epimastigotes and trypomastigotes forms by IFA and Western blot was evaluated. IFA assays were carried out as described elsewhere using a FITC labelled anti-Human IgG conjugate (Sigma). Antigenic extracts of *T. cruzi* and *T. rangeli* epimastigotes and trypomastigotes were electrophoresed on 10 % SDS-PAGE, transferred to nitrocellulose membranes, incubated with patients serum (1:3000) and revealed using a peroxidase labelled anti-Human IgG (Sigma). Reaction development was performed using the ECL kit (Amersham Biosciences). IFA analysis showed an expressive cross-reaction of chagasic serum with *T. cruzi* and *T. rangeli* epimastigotes, independently of the clinical form of the disease. In contrast, a weak reaction was observed for *T. rangeli* trypomastigotes, showing a 2-3 fold titre decrease. Preliminary Western blot analysis revealed with these patients serum revealed distinct antigenic profiles for *T. rangeli* and *T. cruzi* epimastigotes and trypomastigotes.

## IM59 - IMMUNOLOGICAL SCREENING OF Leishmania chagasi GENOMIC DNA EXPRESSION LIBRARY CONSTRUCTED IN BACTERIOPHAGE $\lambda$

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Several *Leishmania* proteins have been identified and characterized for the development of serodiagnostic tests for visceral leishmaniasis (VL). Currently available serological tests, using *Leishmania chagasi* extracts, have low accuracy and reprodutibility, and those using recombinant antigens have variable sensitivities in field tests in Brazil. The use of several recombinant antigens in an assay may increase the assay sensitivity, but care should be taken not to introduce in the assay an antigen that will significantly reduce the assay specificity. A systematic selection of *Leishmania* recombinant antigens, therefore, is crucial for the development of both sensitive and specific tests. In the study described herein, a *L. chagasi* genomic DNA expression library was screened by VL patients' sera and clones of recombinant proteins, potentially candidates to compose a diagnostic test, were obtained. Six VL patients' sera were selected out of twelve, based on the diversity of antigenic recognition in crude *L. chagasi* extract by Westtern Blotting, and pooled. The pooled sera were used to immunoscreen 220.000 clones of a *L. chagasi* genomic library, constructed in $\lambda$ phage by our research group. Among these, 60 were recognized by the pooled sera, and sequencing and characterization of 25 of these are underway. A *L. chagasi* genomic DNA library in $\lambda$ phage contains, therefore, a proportion of *Leishmania* antigen-expressing clones that is large enough to allow their identification by pooled human sera recognizing a large number of antigens in a parasite lysate.

## IM60 - ACTIVATION OF THE KININ PATHWAY INTEGRATES INFLAMMATION TO INNATE AND ADAPTIVE IMMUNITY IN Trypanosoma cruzi INFECTION.

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Increased vascular permeability is a common response to excessive generation of kinin peptides in injured tissues. Recently, we demonstrated that kinins may bridge inflammation to innate and adaptive immunity by inducing the maturation of dendritic cells (DCs). This concept is supported by evidences that activation of the B2 kinin receptor (B2R) in DCs upregulates IL12 production, stimulating type 1-immunity polarization (J.Immunol. 170: 5349-5353, 2003). Here we show evidence that these mechanistic principles occur in the context of Trypanosoma cruzi experimental infection. The dynamics of the inflammatory response evoked by trypomastigotes (topically applied) was studied by intravital microscopy, using the hamster cheek pouch (HCP) as a model. The importance of kinin homeostasis in macromolecular leakage responses (FITC-Dextran) in the HCP was investigated by adding ACE inhibitors and kinin receptor antagonists to the superfusate. Mice (WT, TLR4KO, TLR2KO, B2RKO) were infected with (i): trypomastigotes (ii) trypomastigotes pre-treated with synthetic inhibitors of cruzipain (iii) epimastigotes. Prior to infection, the animals were either treated with (iv) mab RB6 (to deplete neutrophils) or (v) ACE inhibitors (to block kinin-degradation pathways) and/or (vi) HOE 140, a specific antagonist of the B2R. In some experiments, tGPI-mucin and cruzipain were injected, alone or together, in the above mentioned groups of mice. IL-4 and IFN-gamma production by Ag-stimulated spleen cells were determined at various time after infection Analysis of the dynamics of the inflammatory process elicited by trypomastigotes revealed that TLR2 and B2R act cooperatively, intensifying vascular permeability by a neutrophil-dependent mechanism. Analysis of the parasite factors driving the B2R-dependent inflammation implicated tGPI-mucins as the putative TLR2-stimulator and cruzipain, as the kinin-generating protease. Activation of the TLR2-B2R axis, at early stages of infection upregulates IFN-gamma production by T. cruzi-specific T cells. Analysis of innate immunity responses supports the concept that BK-mediates upregulation of IL12 production by stimulating the maturation and recruitment of DCs to the draining lymph node.
Supported by CNPq, MCT, FAPERJ, VW Foundation and REDE TB (Projeto Milênio)

## IM61 - Cross-protective efficacy of a Leishmania donovani-DNA vacine against visceral and cutaneous murine leishmaniasis

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We analised the effect of the purified FML antigen, the recombinant NH36 (rNH36), both in combination with saponin, and of the NH36 DNA vaccine (VR1012-NH36), against the infection by L. (L.) chagasi and L. (L.) mexicana in Balb/c mice. Mice infected with L. (L.) chagasi showed enhanced IgG, IgG1 and IgG2a responses induced by the three vaccines being the FMLSAP the most potent formulation followed by NH36SAP and VR1012NH36, while mice challenged with L. (L.) mexicana, showed a global non-specific enhancement of IgG antibodies. The intradermal reaction to L. (L.) donovani or L. (L.) mexicana antigens was enhanced with either rNH36 or FML-vaccines while mice immunised with VR1012-NH36 reacted only reacted against L. (L.) donovani (p¡ 0.001). Reduction of parasitic load was achieved after FML and NH36 vaccination (79
Support: CNPQ; MCT/PRONEX/FINEP; PRONEX-FAPERJ; FAPERJ; RHAE-CNPQ; CEPG-UFRJ.

## IM62 - Immunotherapy against experimental canine visceral leishmaniasis with the Leishmune vaccine.

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American visceral leishmaniasis, is an important canine zoonosis against which there is not efficient treatment. Seropositive infected dogs are sacrificed for epidemiological control. Recently, we demonstrated the immunotherapic effect of the FML-QuilA and the FML saponin R vaccines (1 mg adjuvant) on dogs experimentally infected with *L. (L.) donovani* and dogs naturally infected with *L.(L.) chagasi* from Araçatuba, SP, when seropositive but still asymptomatic. A strong protective effect was observed characterized by the asymptomatic status, IgG2 predominant antibodies, parasite free, IDR positive and normal proportions of CD4 and CD21 lymphocytes. CD8 proportions were significantly increased as expected for a Quillaja saponin vaccine treatment. In the present work we are analizing the imunotherapeutic effect of the Leishmune$^R$ (1mg saponin) vaccine in 25 mongrel dogs experimentally infected with $2x10^8$ amastigotes of *L. chagasi*. The animals were monitored monthly by the FML-ELISA assay and clinical evaluation. Blood lymphocytes FACS analysis and parasite investigation in bone marrow and peripherical blood samples were also performed. After raise of anti-FML antibodies and mild kala-azar symptoms, 12 dogs were treated with Leishmune$^R$ and 13 with saline. After the first Leishvacin$^R$ dose, vaccinees showed higher 492nm absorbancies (0.751) than saline controls (0.508). However, this difference is not significant (p¿0.05). The parasite investigation disclosed no *Leishmania* neither in blood nor in bone marrow cultures. The means of the FACS analysis showed normal proportions in Thy, CD5, CD4, CD8, CD21 markers before immunotherapy (day 180).

| | THy1 | CD5 | CD4 | CD8 | CD21 |
|---|---|---|---|---|---|
| Saline | 78,50% | 63,23% | 42,43% | 26,86% | 13,73% |
| Vaccine | 80,27% | 64,14% | 45,21% | 27,11% | 13,35% |

At day 210 then, the infection became apparent, the dogs still showed a normal lymphocyte pattern and the serological response indicates the immunopotential of Leishmune$^R$. Support: CNPQ; RHAE-CNPQ, PRONEX/FAPERJ, FAPERJ, Fort Dodge Saude Animal

## IM63 - Serology of subjects with amoebiasis in Governador Valadares, Minas Gerais, Brazil

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The protozoan parasite *Entamoeba histolytica* inhabits the human large bowel and occasionally can invade intestinal mucosa. Diagnostic of amoebiasis is usually done by fecal examination, which cannot distinguish *Entamoeba histolytica* from a similar but non-invasive *Entamoeba dispar*. Invasive amoebiasis is generally associated to high antibody titles and must be treated with drug that acts in the tissue. In contrast, antibody titers are absent or very low in non-invasive infection, whose treatment must utilize luminal amoebicides. We proposed to apply serologic methods to determine potentially invasive amoebiasis and help the treatment indication for the people from two districts of Governador Valadares, Minas Gerais. Techniques of centrifugation

and sedimentation were utilized to select individuals with cysts of *E.histolytica/E.dispar*. After clinical evaluation, sera from infected subjects were tested by ELISA method in order to determine reactivity for IgG anti-*E. histolytica*. Forty-five from 49 subjects tested were non-reactive in ELISA and did not present symptoms. Two from four IgG positive subjects presented symptoms of amebic dysenteric colitis. One of them had vague abdominal pain and the last one was asymptomatic. Our results showed a predominance of luminal infections and indicated a correlation between serology and clinical picture. Metronidazole is the drug usually available for the treatment of amoebiasis in Health Care Centers in Governador Valadares. Since metronidazole has no efficient action against parasites in the intestinal lumen, drugs with luminal action, as paramomicin and diloxamide furoate must be included in therapeutic arsenal against amoebiasis in this area. Apoio: FAPEMIG, CNPq, Laboratório de Amebíase da UFMG, Secretaria Municipal de Saude de Governador Valadares, MG

## IM64 - Comparative study of resistance of IFN gamma -/-, iNOS-/- and wild type mice infected with Leishmania major

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Leishmania major is an obligatory intracellular parasite that lives in macrophage cell lineages. Resistant host immune system produces IFN-g that trigger lots of resistance mechanisms like enhanced phagocytosis and respiratory burst by macrophages, antibody production with IgG switch and others. One of most potent IFN-g activated killing mechanisms is the induction of NO production by iNOS in cell host. This is known to be the most important factor in leishmaniasis resistance. In this study, we investigated the contribution of IFN-g and iNOS in the control of L. major infection. In such purpose, we infected IFN-g -/- and iNOS-/- mice and their C57BL/6 wild type (WT) control mice with 1x106 stationary forms of Leishmania major in footpad. We found that, although iNOS-/- presented earlier lesions (1 to 2 mm in 3rd to 4th week of infection) it stabilized around 2 mm till the end of experiment. However, iNOS-/- presented severe necrosis. On the other hand, IFN-g -/- mice took more time to present lesions, but around 6 weeks of infection they had larger lesions and less necrosis than iNOS-/-. WT mice presented the smallest lesion. IFN-g -/- mice were found to be highly susceptible to infection being the only group to die around 10 to 11 weeks of infection. The other groups, iNOS-/- and WT, were followed by 30 weeks. IFN-g -/- also presented severe tissue parasitism in lesion site, draining lymph node, spleen and liver. Of note, parasitism in liver were around 100 times higher per mg of tissue in IFN-g -/- when compared to WT and iNOS-/-. Despite IFN-g -/- and iNOS-/- mice presenting severe histopathological when compared to WT, IFN-g -/- group presented the most severe lesions in footpad, lymph nodes, spleen and liver. With these results is possible to grade the susceptibility to L. major infection in witch IFN-g -/- mouse was found to be the most susceptible, iNOS -/- mouse as an intermediate and WT mouse as completely resistant. These results suggest IFN-g-dependent and INOS-independent mechanisms of resistance for further investigation.

## IM65 - Study of the effect of atorvastatin on the immune response against Leishmania major infection

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The first commited step to the cholesterol synthesis is catatalysed by the enzyme hidroximetilglutaril-CoA reductase (HMG-CoA). Hence, HMG-CoA reductase inhibitors, or statins, have been found to be useful tools to lower cholesterolemia. Recent data sugest, that statins also may be potent immunomodulatory agents. Atorvastatin (AT) can modulate the immune response by diminishing Th1 and augmenting Th2 responses. L. major infection in C57BL/6 mouse is characterized by polarization towards the Th1 response, which confers resistance. The main purpose of this study was to investigate the effect of AT in the immune response against infection with L. major. C57BL/6 mice were treated with AT (10 mg/Kg/day, per os) beginning at -2 days (ATLm) or 14 days (LmAT) of infection with L. major (1x106 stationary forms in hind footpads). PBS was administered as control. After 10 weeks of infection the animals were sacrificed. Higher parasitism was detected in treated groups (more than 2 log fold increase per mg of lesion). Treated groups also presented higher IFN-g production than PBS-treated group at draining lymph node. No difference was detected in IFN-g levels in lesions among all three groups. ATLm and LmAT expressed larger amounts of mRNA for MCP-1 and smaller amounts of mRNA for RANTES in footpad lesions, when compared to the control group. MCP-1 is a chemotactic factor for macrophages and may be involved in migration of non-activated macrophages to lesion the site, while RANTES is associated with migration of IFN-g-producing T cells and activated macrophages. The balance between MCP-1 and RANTES is known to be important in resistance or susceptibility against L. major. These results show that, although the effects were not dramatic, AT interferes in the modulation of the immune response against L. major infection by altering the mechanisms of control of parasitism.

### IM66 - A histopathological and immunocytochemical study of skin biopsies of dogs naturally infected with Leishmania (Leishmania) chagasi in Belo Horizonte, MG, Brazil

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Canine Visceral Leishmaniasis (CVL) is a zoonosis and a chronic systemic disease of the dog caused by a protozoan of the genus Leishmania infantum in the Old World and Leishmania chagasi in the New World. In this work we describe the prevalence of dogs naturally infected with Leishmania (Leishmania) chagasi. In parallel we have analyzed a chronic inflammatory picture present in canine paraffined tissues. Skin biopsies obtained from ears of a hundred sixty-tree animals infected with Leishmania chagasi (Belo Horizonte, MG) were collected for histopathological and immunohistochemical analysis. For histopathological exams paraffined skin sections stained by Hematoxilin-Eosin (HE) were analyzed by optic microscope in order to evaluate the chronic inflammatory reaction. For immunohistochemistry studies the streptoavidin peroxidase method was carried out for Leishmania detection in canine paraffined tissues (Tafuri et al., 2004). In fact they were easily observed within inflammatory macrophages in skin biopsies. Our results have demonstrated forty-six positive animals (28 percent). The general inflammatory reaction picture was the same for all animals. It was characterized by a diffuse and chronic inflammatory reaction localized mainly in the deep dermis. The cellular exudate was mainly composed by macrophages, plasmocytes and lymphocytes. In this work we have observed that animals with immunohistochemical negative results have a discrete chronic inflammatory reaction when compared with the positive animals. We have concluded that is rare to find negative animals with an intense chronic inflammation. In fact, in this work we find only one animal with an intense chronic inflammation in the dermis associated to intracellular forms of Leishmania parasites. Apoio Financeiro: FAPEMIG, CNPq, UFMG

### IM67 - Response of C57BL/6 b2-microglobulin-deficient mice after Vaccine-Induced Immunity against Leishmania amazonensis Infection

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*Leishmania amazonensis* is an intracellular parasite and have been identified from patients with diverse clinical forms, including cutaneous leishmaniasis and diffuse cutaneous leishmaniasis. Although the role of CD8+ T cells during immunization of mice against *L. major*, little is known for infection with *L. amazonensis*. This study was aimed at evaluating the effects of CD8+ T cells in protection against L. amazonensis challenge in vaccinated mice. The vaccine used was killed *Leishmania amazonensis* promastigotes(Leishvacin), produced by Biobrás. C57BL/6 b2-microglobulin-deficient mice ($\beta 2-M-/-$) and wild type controls, were vaccinated in the base of the tail. Each animal received two inoculations at an interval of seven days, each dose containing 100 mg of protein vaccine plus 250 mg of *Corynebacterium parvum*. Twenty-eight days after the second dose, the animals received a further 10 mg of vaccine. Seven days after this booster, the animals were challenged with *L. amazonensis* in the left hind footpad. This protocol of vaccination protected C57BL/6 mice against infection: these mice showed smaller lesions and smaller parasite numbers than non-vaccinated controls. $\beta 2-M-/-$ mice were not more susceptible to infection than WT. Comparison between vaccinated and non-vaccinated $\beta 2-M-/-$ mice showed a statistically significant difference in lesion sizes at 4 to 15 weeks post infection. At 4 weeks, the levels of $IFN-\gamma$ in lymph node and spleen cell cultures from $\beta 2-M-/-$ vaccinated mice were higher when compared to $\beta 2-M-/-$ non-vaccinated mice. At 15 weeks of infection, vaccinated and non-vaccinated $\beta 2-M-/-$ mice showed similar levels of $IFN-\gamma$ Lymph node and spleen cell cultures from C57BL/6 and $\beta 2-M-/-$ vaccinated mice presented higher levels of $IFN-\gamma$ when compared to non-vaccinated mice. IL-4 was not detected in supernatants from lymph node or spleen cell. Our results show that the control of de *L. amazonensis* infection conferred by vaccination is independent of CD8+ T cells. Support: CAPES and FAPEMIG

### IM68 - Reactivity of sera antibodies from patients with Chagas disease with different clinical forms against *Trypanosoma cruzi* proteins separated through two-dimensional electrophoresis.

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*Trypanosoma cruzi* cruzi antigens used to diagnose Chagas disease and also to discriminate chagasic patients with different clinical forms still need to be identified. In this work, the reactivity of chagasic patients' sera with different clinical forms against *T. cruzi* proteins separated by two-dimensional (2D) electrophoresis has been investigated. Proteins were obtained from epimastigote forms of *T. cruzi* Y strain, in exponential growth phase in LIT medium and resuspended

in lysis buffer. Soluble proteins were fractioned in a 2D electrophoresis. In the first dimension, proteins were separated by isoelectric focusing, non-linear pH 3-10 and in the second dimension by SDS-PAGE 12%. After the electrophoresis, 4 gels were transferred to nitrocellulose membranes and one gel was silver stained. Membranes were incubated with a sera pool from 5 chagasic patients in the clinical forms Indeterminated (I), Cardiac (C), Digestive (D) and not infected (N). The membranes were further incubated with a second antibody anti-human total IgG conjugated with alcaline phosphatase revealing protein spots. The spots silver stained and those recognized by sera were analyzed in the PDQuest software. The silver stained gel presented 290 well-defined spots. The spots recognized by each clinical group of patients' sera were 55 by D, 52 by I, 40 by C and 36 by N. The comparison of the membranes of the four groups allowed us to determine how many and which spots were clinical form specific. Group D presented 10 (18.2%), group I, 7 (13.5%) and group C, 3 (7.5%) specific spots. These specific proteins are being identified by mass spectrometry. In conclusion, 2D electrophoresis followed by western blot and mass spectrometry constitutes a good option to locate and identify candidate proteins specific for each clinical form of Chagas disease. Financial support: PDTIS/FIOCRUZ, CPqRR/FIOCRUZ and CNPq.

## IM69 - A NOVEL PROTOCOL FOR THE STUDY OF MECHANISMS INVOLVED ON PS EXPOSURE BY *Leishmania amazonensis* AMASTIGOTES OBTAINED FROM *IN VITRO* MOUSE INFECTED MACROPHAGES

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The exposure of phosphatidylserine (PS) on the surface of apoptotic cells is important for the clearence of these cells without the induction of an inflammatory response. Previous work from our group demostrated that the parasite *Leishmania amazonensis* (*LLa*) uses the same mechanism to increase macrophage permissivity to infection. PS recognition on parasite surface by macrophage results in increased production of TGF-$\beta$ and decreased production of nitric oxide by infected cells. The mechanism of PS exposure on the parasite surface and its eventual modulation by host macrophages is not well understood. With the aim of tackling this question, we developed a protocol for the purification of viable parasites from the parasitophorous vacuole of *in vitro* infected macrophages. Infected cells were scrapped from culture flasks and lysed in a tissue homogenizer, in the presence of protease inhibitors. After three washes in Phosphate Buffered Saline (2700 rpm, 17 min., 4oC) the cells were ressuspended in Dulbecco's Modified Eagle Medium containing 4% Fetal Calf Serum and incubated for 1 h in a shaker at 34oC and 50 rpm agitation. The cells were washed again in PBS for three times and prepared for cytometric analysis. Results show that isolated amastigotes are positive for mAb 2A3-26, which specifically recognizes amastigote forms of *LLa*. After purification, the rate of parasite death is of about 10% as seen by propidium iodide staining. Amastigotes are negative for $\alpha$-CD14 antibody, indicating that there are no macrophage membranes on their surface. This was confirmed by transmission electron microscopic analysis. Preliminary results obtained with this protocol suggest that PS exposure by the amastigotes depends on the degree of macrophage activation and on the nature of the activating cytokine.

## IM70 - Trypanosoma cruzi infection sensitizes mice to LPS-induced shock: unraveling the mechanism

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T. cruzi infection induces severe apoptosis of lymphocytes and granulocytes, a phenomenon that has been linked to deactivation of macrophages and is thought to impair T. cruzi clearance. Paradoxically, T cruzi infection sensitizes to LPS-induced shock, leading to a hyperproduction of TNF in response to LPS in vivo, paralleled by increased production of TNF in response to LPS by macrophages from infected mice in vitro. In a primary effort to solve the paradox, we studied the basis of T. cruzi Y strain sensitization to LPS. High numbers of trypomastigotes administered along with LPS did not cause shock - an interval of infection was required to sensitize mice. TLR-2 -/- mice, which do not recognize GPI-mucins, a major inflammatory component of T. cruzi, were sensitized to LPS by T. cruzi infection. Also, neither GPI-mucins (10mg) nor T cruzi (107 blood trypomastigotes) administered along with the hepatotoxin D-gal lead to toxic shock, while LPS (10mg) + D-Gal controls succumbed to shock. MIF -/- and TNF-R1-/-, which are resistant to high doses of LPS and LPS+D-gal, respectively, were sensitized by T cruzi infection to low doses of LPS. We have failed to either prevent or exacerbate T. cruzi-induced sensitization to LPS by transferring apoptotic, necrotic or live splenocytes i.v. one day after or i.p. 5 days before sensitization. Together, our data indicate that the sensitization induced by T. cruzi infection to LPS is not caused by immediate inflammatory synergism, but rather depends on events on the course of infection. Induction of apoptosis cannot be discarded as one of these factors so far.
Financial support: CNPq, FAPERJ, FUJB, FIOCRUZ and Pronex.

## IM71 - Apoptosis in macrophages infected with *Leishmania amazonensis*

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Macrophages infected with *Leishmania* eventually rupture releasing amastigotes, which are infective for neighboring cells. Being an amplifying step, the macrophages death may be a key point in the development of leishmaniasis. In the present work, we monitor the death of *L. amazonensis*-infected BALB/c peritoneal macrophages in vitro, investigating whether they die through apoptosis. Using the MTT assay, we have observed a sudden reduction in the viability of the infected macrophages, as compared to non-infected cells. In order to investigate whether the cells were dying through apoptosis, we initially examined the fragmentation of the DNA, using both agarose gels and the TUNEL technique. In agarose gels, we have observed that, in 24 hs after infection, *L. amazonensis*-infected cells presented a DNA fragmentation that appeared as a ladder pattern with fragment sizes multiples of 200 bp, typical of apoptotic cells. The fragmentation of macrophages DNA were confirmed using the TUNEL technique, which allow us to observe that, also in 24 hs after infection, around 40% of the macrophages had their nuclei labeled. These results suggests that programmed cell death occurs in macrophages infected with *L. amazonensis* in vitro. Therefore, it is possible that apoptosis contribute for the progression of the lesions in cutaneous leishmaniasis.

## IM72 - IN VITRO ASSAY OF CANINE PERITONEAL MACROPHAGES BINDING TO LEISHMANIA (Leishmania) chagasi.

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Visceral Leishmaniasis is a zoonosis and dogs represent the principal domestic reservoir of the disease. In the New World the ethiological agent of the disease is Leishmania (Leishmania) chagasi which is transmitted by the phlebotomine Lutzomyia (Lutzomyia) longipalpis. The Leishmania promastigote-macrophage interaction occurs based on multiple receptors presented on the biological membrane surfaces. The success of the parasite infection is dramatically dependent of this early interaction in the vertebrate host and it will permit or not the development of the disease. The major event of these systems is the binding of serum complement opsonized promastigotes to macrophage receptors for complement. Complement-dependent opsonization of parasites not only improves their adhesion to macrophages, but also enhances their intracellular survival. This interaction is mediated by the cell surface receptors from the family of integrins CR3 (CD11b/CD18) and C3bi fragment of complement mainly. In this study, we have carried out a binding assay method to study the interaction between Leishmania (Leishmania) chagasi and peritoneal macrophages from dogs presenting different clinical forms of the disease. To study parasite-macrophage interaction, peritoneal macrophages were obtained from 31 dogs (8 asymptomatic, 8 symptomatic, 8 oligosymptomatic and 7 control dogs) and adjusted to 3 x 106 cells/mL . Leishmania (Leishmania) chagasi parasites (stationary-phase) were adjusted to 5x107 cells/mL. The Leishmania-binding assay was performed in a 24-well plate during 45-60 minutes at 35°C over coverslips. We carried out assays in the presence of normal serum or in the presence of a final concentration of 5 percent of C5 deficient (serum from AKR/J mice) mouse serum. It means that the reaction was performed in the presence or absence of C3bi fragment of complement. The Leishmania parasites were used in crescent concentrations to evaluate this interaction. Then, the number of infected macrophages was counted in a optical microscope, as well as the number of parasites per macrophages in these two experiments (dependent serum and independent serum). The results demonstrate that the number of parasites bound to macrophages was dramatically increased in the serum dependent group (with C5 deficient serum mouse) in all experiments, with no differences among the clinical groups, including control dogs. These results could indicate that C3bi complement fragment is really important to enhance the parasite phagocytosis by canine macrophages. Also, there was no difference considering the defined clinical animals. It seems that macrophages from these groups can react similarly when stimulated by complement and Leishmania (Leishmania) chagasi, "in vitro".
Supported by: CNPq, FAPEMIG, UFMG

## IM73 - HOST GENETIC BACKGROUND IS NOT ESSENTIAL IN THE PROTECTIVE IMMUNITY AGAINST *Trypanosoma cruzi* PROVIDED BY IMMUNIZATION WITH *Phytomonas serpens*, A TOMATO PARASITE

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Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, has quite a variable clinical presentation, ranging from asymptomatic to severe chronic cardiac and/or gastrointestinal disease. The reason for that is not completely understood, but both parasite and host genetic traits are certainly involved. We previously demonstrated that *Phytomonas serpens*, a tomato parasite, shares antigens with *T. cruzi*. These antigens are recognized by human sera and induce protective immunity in Balb/c mice. We then decided to turn our attention to the role of host genetic background. To study this, we compared the infection of three lineage of mice, two inbred (BALB/c and C57BL/6) and one outbred (Swiss), with *T. cruzi*(Y strain). A reduction in parasitemia and increase in survival was observed only in Balb/c and C57BL/6 infected mice that were previously immunized with *P. serpens*, when compared to non-immunized mice. All immunized Swiss mice died until 18th day post infection. Our results indicate protective immunity against *T. cruzi* provided by oral immunization with *P. serpens* is not controlled by the genetic background of the mouse. Since BALB/c have the H-2 haplotype H-2$d$ and C57BL/6 does H-2$b$, it is pos-

sible that other factors and not MHC variability may be involved in this process. In addition, Western blot analyses with serum from immunized inbred and outbred mice evidenced a great difference between the recognition patterns to *T. cruzi* antigens. Supported by CNPq and CPG/UEL

## IM74 - ANTIBODY RESPONSE PROFILES INDUCED BY Plasmodium falciparum GLUTAMATE-RICH PROTEIN (GLURP) IN A NATURALLY EXPOSED INDIVIDUALS FROM BRAZILIAN ENDEMIC AREA.

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The Glutamate-Rich Protein (GLURP) is an exoantigen considered to be one of the leading malaria vaccine candidates. In this study we evaluate the antibody response profile induced by GLURP in naturally exposed individuals from Brazilian endemic area (Rondonia State). The IgG response against recombinant proteins (R0 and R2 regions) and synthetic peptides (R0: P3, P4, P5, P8, P9, P10, P11, S3; R2: S4) corresponding to different regions of the protein was evaluated by ELISA and the HLA-DR and DQ were determined by PCR. Anti-R2 antibodies were significantly more prevalent (79%) than anti-R0 (67%). IgG responses to both antigens were associated with age and exposure. The frequencies of response to P11 (49%) and S4 (53%) were greater compared to the other peptides. P11, S3 and S4 showed higher levels of IgG responses than did P3, P4, P5, P8 and P10. The absence of anti-R0 response was associated with HLA-DR11 and -DQ7 and the absence of anti-R2 response was associated with HLA-DR12. We also observed positive and negative associations between HLA and the immune response against GLURP-derived peptides (positive: P3 with DR4 and DQ8; P4, P8 and P9 with DR13; P10 with DR8; P11 with DR8 and DQ4; negative: S4 with DR7). Our data suggest that the R2 is the immunodominant region of the GLURP and that S4 and P11 are the immunodominant B-cell epitopes in individuals from our Brazilian endemic area. Our results also suggests that there are significant associations between HLA-DR and -DQ and the IgG response against GLURP. Given the increasing focus on the use of subunit vaccines in the control of malaria, the concern of the influence of class II allele frequencies in ethnically diverse populations may be important before vaccine trials are conducted among people naturally exposed to parasites. Supported by FIOCRUZ, CNPq and FAPERJ

## IM75 - *PLASMODIUM BERGHEI* ANKA INFECTION IN THE SWISS WEBSTER MOUSE: A NEW MURINE MODEL FOR IMMUNOPATHOLOGY OF CEREBRAL MALARIA

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**Introdution**: Murine models are largely used to understand the mechanisms of immunity and pathology in malaria. Here we describe a new model of cerebral malaria (CM) using the outbred mouse strain Swiss Webster. **Methods and Results**: Two experiments were conducted with a total of 32 mice inoculated with $1x10^6$ *P. berghei* ANKA-parasitized red blood cells. Mice presenting clinical signs of CM (convulsions, roll-over, paralysis or coma) were killed and organs were collected in formalin, processed and slides stained with hematoxylin-eosin or Giemsa. Mice presented parasitemia above 10% on day 5, and 21 (65%) mice showed clinical signs of CM between days 6 and 10 of infection. The brain presented hemorrhages, oedema and the vessels were plugged with monocytes. In the lungs, the alveoli walls presented mononuclear cell infiltrates in different degrees. In the liver, hepatocytes were vacuolated and mononuclear cell infiltration in the synusoids and pigment were observed. Intense monocyte adherence was observed in the lung and liver vessels. In the thymus, intense apoptosis was observed in the cortical area, ranging from starry-sky appearance to almost complete depletion of thymocytes. Mast cells, eosynophils and myelopoiesis were observed in the cortex. The spleen presented marked changes with disturbance of the white pulp structure, especially the germinal center, with intense centroblast proliferation - without centrocyte differentiation - and apoptosis in the follicles. Marginal zone disappeared and there was an overwhelming plasmacytogenesis. The red pulp was hyperthophic. **Conclusion**: Understanding the mechanisms leading to disturbance of the lymphoid microenvironments must be necessary to understand immunity and immunopathology in malaria. Financial Support: IOC/Fiocruz

## IM76 - Modulation of the host macrophage anti-inflammatory activity by phosphatidylserine on the surface of amastigotes of *Leishmania (L) amazonensis*.

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*Leishmania (Leishmania) amazonensis (LLa)* is one of the ethiologic agents of cutaneous leishmaniasis in the New World. Amastigote forms are responsible for the progression and spreading of infection in the vertebrate host. They invade and proliferate inside host macrophages, by means of escape mechanisms from the leishmanicidal activity of these cells. We have shown that amastigote forms of *LLa* expose phosphatidylserine (PS) at their surface. This phospholipid acts as a ligand for a specific receptor on phagocyte surface, participating on amastigote recognition and internalization (Balanco and Moreira *et al. 2001*). (BALB/c x C57Bl/6) F1 mice infected by high PS-exposing parasites showed higher footpad lesions when compared to the ones infected by low-PS amastigotes, confirming previously *in vitro* results. Macrophages infected by high PS-exposing amastigotes produce larger amounts of TGF-$\beta$1 than macrophages infected with low PS-exposing parasites. This result suggests that PS recognition is essential for an anti-inflammatory activation of macrophages. Furthermore, amastigotes purified from interferon-$\gamma$ (IFN$\gamma$) receptor knockout mice in a 129/J background exposes less PS than the wild-type (WT) mice, although knockout mice develop a significantly higher non healing lesion when compared to the WT counterpart. These results confirm PS participation on macrophage infection by amastigote forms of *LLa* and suggest a role for macrophage activation by IFN$\gamma$ on the modulation of PS exposure by amastigote forms.

## IM77 - Impaired leucocyte recruitment in T. cruzi-infected monocyte chemoatractant protein 1 (MCP-1) -deficient mice

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Chagas' disease is an inflammatory condition caused by the intracellular protozoan parasite Trypanosoma cruzi that affects 18 million people in Latin America. The chemokine MCP-1 is important to activate macrophages to trypanocidal activity, and is secreted in response to parasite GPI-mucins, a major inflammatory component of T. cruzi, promoting leukocyte recruitment and severe apoptosis of lymphocytes and granulocytes. Herein, we analised the role of MCP-1 chemokine in T. cruzi infection using MCP-1 knockout mice (MCP-1-/-), emphasizing tisular lesions and leukocyte recruitment to infected sites. MCP-1-/- mice were more susceptible to T. cruzi infection, leading to earlier mortality and presenting higher peaks of parasitemia than wild-type controls. The production of IFN-g, however, was higher in MCP-1-/- mice than in wild-type controls. The deficiency in MCP-1 prevented the infiltration of Mac-1+ Mac-3+ cells in the heart, a phenotype associated with recently-recruited macrophages. Also, macrophages from MCP-1-/- mice presented lower NO production and trypanocidal activity than wild-type controls in vitro. The presence of CD8+CD69+ cells was reduced in their hearts and livers, but normal percentages of CD4+CD69+ cells were found. These results confirm the role of MCP-1 as an important recruiter of trypanocidal cells to infected sites.
Financial support: CNPq, FAPERJ, FUJB, FIOCRUZ and Pronex.

## IM78 - Lesion development in *Leishmania amazonensis* infected C57BL/6 mice is influenced by the infection route

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Unlike what is observed in *Leishmania major* infection, C57BL/6 mice don't spontaneously cure *L. amazonensis* lesions. Our ongoing studies aim to understand the aspects of this susceptibility. We have analyzed the *L. amazonensis* infection in C57BL/6 using different inocula ($10^7$ stationary phase promastigotes - SP and $10^4$ metacyclics promastigotes - MP) and different infection routes (footpad and dorsal skin). The kinetics of infection, parasite load, cytokine and serum antibody levels were verified. Although appearance of a lesion at the inoculation site was delayed in mice infected with MP ( 6 weeks), the lesions caused by SP and MP attained similar size by the 9th and 15th week after inoculation, respectively. The parasites were more numerous in the lesions and in the popliteal draining lymph nodes from mice injected with SP. Of note is that C57BL/6 mice infected in the dorsal skin developed a single severely ulcerated lesion and its draining lymph node cells produced less IFN-g than the draining popliteal lymph node of a similarly infected paw, specially when SP were inoculated. In addition, high serum levels of *Leishmania*-specific IgG2ab could be observed in mice infected with SP in the footpad. Our experiments showed that the type and development of the cutaneous lesion caused by infection with *L. amazonensis* in C57BL/6 mice is primarily dependent on the route of inoculation (infection). The progressive and ulcerating nature of the dorsal lesions may be related to lower local IFN-g production and to the anatomic characteristics of the dorsal skin as compared to the footpad skin. Moreover, *L. amazonensis* infection in C57BL/6 mice reaches the systemic circulation and disseminates to the spleen and contra lateral lymph nodes.
Supported by FAPESP

# Vetores - Vectors

## VE01 - Evidences of feeding preference of Triatoma infestans for immune chickens

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Chagas disease affecting 18 million people in the Western Hemisphere is transmitted by the triatomine vector, its main form of propagation. Although much work has been carried on aiming at the development of new drugs for treatment of Chagas disease, it continues to be considered as incurable. There is no vaccine available for preventing Chagas disease. In this work we immunized poultries with proteins from the saliva of *Triatoma infestans*, the main bug transmitting *Trypanosoma cruzi* to man, in Brazil, and we evaluated whether these immunizations produced alterations in some parameters related to the insect physiology. In this regard, we followed up four *T. infestans* generations to determine ratio of survival, fecundity, fertility, duration of period of time required for nymph development and, in addition, the influence of immunizations on the insect feeding habit. The results showed the immune response of the hosts , which turned the host attractive to the insect bite, also influenced its development thus, diminishing the time lapse required for the insect to reach reproductive, adult life. Although these findings contrast sharply with those reported by some authors, but they are in keeping with some others in the literature. Therefore, we believe that additional experiments should be undertaken in order to confirm or to discard the possibility of preventing the insect-transmitted Chagas disease, by curtailing hematophagy through inactivation of bioactive proteins in the saliva of the triatomine bug.

## VE02 - Some aspects on the biology of Lutzomyia intermedia (LUTZ E NEIVA, 1912) related to its vectorial competence, under experimental conditions.

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**Lutzomyia intermedia** is considerated as an important vector of **Leishmania(Viannia)braziliensis** in southeast Brazil. Since the beginning of the last century many papers have tried to correlate the habits of this important sand fly species with is vectorial competence. Among the criteria proposed by Killick-Kendrick (1990) to incriminate a sand fly as a vector of leishmaniasis, the capacity to transmit the parasite in experimental conditions should be investigated. The goal of the present study was to evaluate the capacity of **L.intermedia** to take more than one bloodfeeding, in laboratory conditions, a fundamental aspect for **Leishmania** transmission. Specimens from a colony of **L.intermedia** maintained in Laboratory of Vectors of Leishmaniasis, Dept. of Entomology/IOC according to procedures proposed by RANGEL et al.(1985) and WERMELINGER et al.(1987) methodologys, were used for this investigation. A total of 132 females (F1 generation) were put to feed on human blood through a chick skin membrane feeder. Temperature and humidity conditions, near to 25$^o$C and 80%RH, were under control. Overall, it was obtained 76 fed females, whose laid eggs on the walls of the cage. Seven days after the first feeding, a hamster was offered as a source of a second bloodmeal. Eleven females (14,5%) became engorged, and a second oviposition could be observed. Fourteen days after the first feeding, seven survivor females were able to accepted a third bloodmeal. These preliminary results suggest that non-infected **L.intermedia** females are able to take more than one bloodmeal in captivity.

## VE03 - Effects of the infection of *Babesia bovis* (Babés, 1888) and *Babesia bigemina* (Smith & Kilborne, 1893) on the biological parameters of the tick *Boophilus microplus* (Canestrini, 1888)

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The biological behavior of modified strains of *Babesia bovis* and *Babesia bigemina* and their effect on biological parameters of engorged female of *Boophilus microplus* were studied in eight cattle not infected with *B. bovis* and *B. bigemina*. The unsplenectomized cattle bh1, bh2, bh3 and bh4 were used to maintain free and infected groups of *B. microplus* with unmodified *B. bovis* and *B. bigemina* from Ibiruçu-SP farm, Brazil. The cattle bh5 was infected with unmodified strains of *B. bovis* and *B. bigemina*. In order to obtain engorged female of *B. microplus* infected with *B. bovis* and *B. bigemina*, bh6, bh7 and bh8 cattle were used. The females that naturally dropped from the host were collected, selectioned in groups of 100 and incubated at $27 \pm 2^o$C with more than 80% humidity. The behavior and infections of *B. bovis* and *B. bigemina* were evaluated in hemolymphs, gut content and ovaries of ticks stained with Giemsa. The biological parameters of the free life phase of the engorged females infected with unmodified strains of *B. bovis* and *B. bigemina* were significantly changed. In this group, it was observed an increase in preoviposition period 3.98 days and in residual weight of the ticks, as well as a reduction of oviposition period 7.86 days, weight of posture 9.72 mg, number of eggs 194.47, index of eggs production 3.97%, reproductive efficiency index 98% and real conversion index. The unmodified strains of *B. bovis* and *B. bigemina* are infective and induce significant changes in the biological parameters of the engorged female of *B. microplus*.

## VE04 - Description of *Lutzomyia intermedia* and *Lutzomyia migonei* naturally infected by *Leishmania braziliensis* in Rio de Janeiro revealed by PCR ASSAY

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*Leishmania (V.) braziliensis* is the species responsible for American Cutaneous Leishmaniasis in Rio de Janeiro, where the transmission occurs frequently by *Lutzomyia intermedia* sand flies (genus *Lutzomyia*). In this work we proposed a methodology for the detection of DNA from *Leishmania (Viannia)* in sand flies, by using a PCR multiplex with two primer pairs, each one specific for either of the genus (*Lutzomyia and Leishmania*). The PCR assay was associated with non-radioactive hybridization detection. The amplicons were first analyzed by agarose gel and submitted to hybridization with a biotinylated probe specific for the subgenus Viannia, revealed by an enzymatic assay. The proposed methodology was validated, by using female sand flies fed with reconstituted blood containing infective promastigotes of *L.(V.)braziliensis*. We performed the analysis of insect samples collected in areas of the Rio de Janeiro city, with recent cases of human and canine leishmaniasis. Among the samples collected in the period March to December 2003, *L.intermedia* was predominant (78.34%), followed by *L.migonei*(12.9%), *L.longipalpis* (8.29%) and *L.cortelezzi* (0.69%). The female samples (n=400) were divided into groups of ten individuals each, from the same species (n=40). The groups were composed of *L.intermedia* (n=32), *L.migonei* (n=5), *L.longipalpis* (n=2) and *L.cortelezzi* (n=1). Positive PCR results were obtained only with *L.intermedia* (5/32) and *L migonei* (3/5) collected in Pau da Fome and Colônia Juliano Moreira. In both localities, the infection index for each species did not present a statistically significant difference (p=0,8379). Considering a total of eight infected groups (8/40) and that at least one insect was found positive in each group, it was possible to infer an infection index of 2% for the evaluated specimens. This study represents the first molecular approach to detect natural infections caused by *L.braziliensis*in both *L.intermedia* and *L.migonei* sand fly vectors in Rio de Janeiro. Supported by CAPES/FAPERJ/CNPq.

## VE05 - The Role of Eicosanoids on Yolk Protein Uptake in *Rhodnius prolixus*

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The participation of eicosanoids and second messengers on the regulation of endocytosis by *Rhodnius prolixus* ovaries was investigated using the uptake of *Rhodnius* heme binding protein (RHBP) as experimental model. The rate of RHBP uptake decreased up to 40% in the presence of BWA4C and NDGA, 5 and 12-lipoxygenase inhibitors, respectively, suggesting the involvement of lipoxygenase products on endocytosis regulation. Addition of Leukotriene $B_4$ ($LTB_4$, one product of 5-lipoxygenase pathway) increased *in vitro* the uptake of RHBP by 30%. The content of cAMP in the *Rhodnius*' ovaries was monitored after treatment with different eicosanoids and inhibitors of eicosanoids synthesis. The amount of cAMP decreased in the presence of indomethacin (by 50%), while treatment with $PGE_2$ induced an increase of 85% of this messenger in the ovaries. The presence of $LTB_4$ in the medium inhibited in 60% the content of cAMP in the ovaries, while BWA4C induced a 100% increase of this messenger in the ovaries. Addition of 1$\mu$M DBcAMP in the medium resulted in 30% decrease in the rate of RHBP uptake. Using an antiboby raised against human lipoxygenase we were able to show immunoreactivity in Western Blot for *R. prolixus* ovary protein extracts and we also verified immunostaining in ovarian follicle sections. Taken together these data show that cyclooxygenase and lipoxygenase products participate in the control of protein internalization by modulation of cAMP levels.

## VE06 - *Anopheles darlingi* population genetic by mtDNA sequencing

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*Anopheles (Nyssorhynchus) darlingi* Root 1926 has historically been considered the most important malaria vector throughout South America. In Brazil, *An. darlingi* is the main vector of malaria, especially in the Amazon region, where more than 97% of all cases in the country occur. It is one of the most anthropophilic and endophagic anopheline in the Americas, factors that obviously account for of its potency as important malaria vector. Its distribution in this region is large and recent data has been showed heterogeneity, so much genetics as for the habit. In spite of these reports, few comparative studies of *An. darlingi* populations have been undertaken. The present study is being carried out with populations of *An. darlingi* from Porto Velho. Host-seeking *An. darlingi* females were captured when feeding on humans or when resting on inner houses walls at Portuchuelo, Santo Antônio, Vila Candelária and Batestaca around Porto Velho

in the state of Rondônia. Population genetics is being carried out using mtDNA sequences. mtDNA is maternally inherited and mtDNA variants segregate rapidly between generations leading to relatively high rates of polymorphism. From these sequence data one can recover both rates of gene flow and phylogenetic relationships between populations. The goal of the present study is to use the sequence of genes COI and ND4 to analyze intra-specific polymorphism and establish some relation between molecular markers and the habit and distribution of these mosquitoes populations. Data of mtDNA sequencing has been showed to be very polymorphic for this species. It is also possible to observe the influence of human population on *Anopheles darlingi* distribution.

## VE07 - Sugar digestion: characterization of involved enzymes and influence upon *Anopheles aquasalis* (Diptera:Culicidae) mosquitoes longevity and fecundity.

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Insects have carbohydrates as an important nutrient in their diet, and these substances play a role in life maintenance in most species. In adult mosquitoes, both male and female feed constantly on sugar obtained from nectar of flowers, which constitutes an essential part of the diet of these insects. Despite the use of blood meal proteins in eggs development, ingestion of sugar is necessary for some metabolic needs. Moreover, the presence of carbohydrates in the diet provides an important source of energy for flight, also contributing to increase the prevalence and fecundity. $\alpha$-Glucosidases (EC 3.2.1.20, $\alpha$-glucoside glucohydrolase) compose a group of exo-acting glycoside hydrolases, which catalyze the hydrolysis of $\alpha$-glucosyl residues from the non-reducing end of $\alpha$-linked substrates to release $\alpha$-glucose. These enzymes are probably responsible for the oligosaccharide digestion in the mosquito. The presence of $\alpha$-glucosidases in mosquitoes have previously been detected in the salivary glands of adult *Aedes aegypti*, *Aedes albopictus* and *Anopheles darlingi*. In the present work we discuss the importance of a sugar meal in mosquitoes prevalence and fecundity. A meal composed by blood + 10 % sucrose was the most efficient for both fecundity and prevalence. Absence of blood meals (sugar meal, only) was well efficient in mosquitoes prevalence, and interestingly when only blood was offered, the longevity was the lowest, and the fecundity insignificant, although they had a relatively great laying. $\alpha$-Glucosidases were identified and characterized, both in males and females adult mosquitoes, and the properties of these enzymes were compared. Apparently, in females there were at least three $\alpha$-glucosidases, and two of them were present both in soluble or membrane bound samples. In males, a preliminary biochemical profile revealed that the peak of activity occurs at the same time it occurs in females. However some differences were found in the biochemical properties of these enzymes.

Supported by: FAPESP

## VE08 - Trying to understand cell death and regeneration in adult female *Culex quinquefasciatus* midgut.

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We have previously described the occurrence of apoptosis in *Cx. quinquefasciatus* digestive cells during blood digestion. Later in the digestive process, several regenerative cells are activated and differentiate to columnar, digestive cells.
Considering that these facts were never described in adult, uninfected mosquitoes, we have been investigating their occurrence in other species, i.e. *Anopheles darlingi* and *Aedes aegypti*, confirming that these animals did not present cell death or regeneration during their blood digestive processes.
In order to verify whether the observed occurrence of cell death was due to the toxic effect of mammalian blood (*Culex* genus have ornitophagic behaviour), we compared tissue from mosquitoes fed with avian (chick) or mammalian (mouse) blood, sera or albumin solution. Intestinal cell death and regeneration were observed only in mosquitoes fed with blood (mammalian or avian).
Toxicity of heme generated after haemoglobin hydrolysis should be considered as potentially deleterious. Blood-feeding insects have developed different mechanisms to protect their intestinal epithelia of this threat. *Rhodnius prolixus* converts heme into insoluble haemozoin (Oliveira *et al.*, FEBS Lett., **477**: 95, 2000). In *Ae. aegypti*, heme is bound to the peritrophic matrix (Pascoa *et al.*, Insect Biochem. Mol. Biol., **32**: 517). In addition, we observed that *An. darlingi* presents coarse particles of electrondense material beside the peritrophic matrix, probably heme particles. *Cx. quinquefasciatus* did not present any evidence of some kind of detoxification, suggesting that this could be the cause of apoptosis and subsequent cell regeneration.

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## VE09 - Initial characterization of *Culex quinquefasciatus* vitellogenesis.

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*Culex quinquefasciatus* is a cosmopolitan, domestic, and highly antropofilic mosquito. Despite its importance as a nocturnal bother and a cause of allergies, this species is largely neglected, perhaps because it is not a vector of important human endemies as occurs with the other Culicinae members. In order to improve our knowledge on the physiology of this animal, putative vector of the emergent West Nile Virus, we present here a preliminary investigation of its vitellogenesis.
Putative vitellogenin subunits, around 210 and 90 kDa, are detected 24 h after blood feeding (abf). These proteins are no longer present in haemolymph 72 h abf while still increasing

in the ovaries samples. Another polypeptide doublet of 57-60 kDa is present only in ovary samples, more abundant at 48 h abf.
Ovarian follicles of adult females contain a cluster of cells surrounded by a layer of epithelial cells. The oocyte is distinguishable by the presence of small lipid droplets in its cytoplasm. This aspect persists during all the development process, which is marked by the accumulation of yolk platelets. Both the inclusions only leave a ring of empty cytosol at the oocyte cortex.
Broad channels between epithelial cells appear soon after blood feeding, hypothetically to permit the contact of haemolymph and oocytes. Another space, encircling the oocyte, is quickly filled with small, dark globules, secreted by the epithelial cells, which grow and fuse to form the endochorion. No space is formed between epithelium and nurse cells, located at one of the oocyte poles.
AFC is a FAPESP fellowship.

## VE10 - Humoral immune response against *Dipetalogaster maximus* (Reduviidae; Triatominae) saliva among non-chagasic individuals in Baja California, Mexico

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*Dipetalogaster maximus* is an endemic species in Baja California found among rocks associated with lizards and possibly with mammals. Some inhabitants from La Paz (Mexico) present cutaneous reactions to *D. maximus* bites due to penetration of the insect on human dwellings to feed, without intradomiciliary infestation. To better understand the interaction between this triatomine species and human hosts, the purpose of this work is to measure antibodies against *D. maximus* salivary components in individuals from the village of La Paz, Baja California, Mexico. ELISA was used to determine immunoglobulin subclasses distribution among 71 non-chagasic individuals. The results showed predominance of IgG2 (24/71) followed by IgG1 (21/71), IgG4 (18/71) and IgG3 (3/71). Nineteen individuals showed high absorbance values for at least two subclasses. This profile was not observed in individuals living in *D. maximus*-free areas. The data suggests that antibody trials could be useful to determine levels of human exposure to triatomines, even in areas where there are no domiciliated insects. Considering the successfully initiatives against Chagas disease by eliminating of domestic and peridomestic triatomines species, the study of naturally acquired humoral immune response in populations exposed to non-domiciliated triatomine bites could also be an effective way to determine the human risk to Trypanosoma cruzi infection in low endemic and/or recent human colonization areas.
Financial support: CNPq, Fapemig, ECLAT (CDIA)

## VE11 - The blood feeding effect on the fat body of the mosquito Aedes aegypti.

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The fat body is a multifunctional organ that varies its structure according to the insect developmental stages producing and storing components to be released in the haemolymph. The objective of this work is to investigate the structure of the fat body related with the blood feeding. We used newly emerged and adult females with different ages and feeding conditions (sugar or blood). The abdominal fat body was fixed, dehydrated, embedded in Historesin and sections obtained for toluidine blue staining. Histochemical stains such as Bromophenol blue for protein, PAS for carbohydrate and Feulgen reaction for DNA were also done. In addition, some samples were processed with osmium tetroxide impregnation for lipid detection. Samples also were processed for analysis in the scanning electron microscope. Fat body of newly emerged females and females without blood diet is formed by lobes lined by aggregations of globular cells and covered by a basal membrane with irregular surface. The fat body of blood fed females form flat sheets adjacent to integument and became smooth. The histological sections of the fat body revealed a heterogeneous organ formed by two types of cells: large number of trophocytes and few oenocytes. Trophocytes have an excentric nucleus and a strong PAS-positive cytoplasm while the oenocytes have a central nucleus and weakly PAS-positive cytoplasm. Trophocytes have two types of inclusions: one bigger and osmiophilic with great number and other smaller in lesser amount, which are bromophenol blue positive. After aging and blood feeding, size and number of trophocyte osmiophilic inclusions increase, probably due to nutrient rich diet that probably increases the cell storage. Furthermore, nucleolus increases in blood fed females, suggesting increase of synthesis activity during gonotrophic cycle. Oenocytes locate preferentially in the lobes periphery. They have central nucleus and strong basophilic cytoplasms that are uniformly stained by bromophenol blue.

## VE12 - *Aedes aegypti*: Analyses of Genetic variation in Brazil based on mitochondrial DNA sequences

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*Aedes (Stegomyia) aegypti*(L.) is the principal vector of a large number of debilitating or lethal human arboviruses, including dengue and yellow fever. There are many components associated to the epidemiologic and transmission cycle of these and other arboviruses transmitted by *Aedes aegypti*. Dengue is a mosquito-borne endemo-epidemic viral disease, caused by any one of the four dengue serotypes. It is a major health problem in terms of morbidity and mortality in many tropical regions of the world. Over 50 million cases of dengue

fever and dengue hemorrhagic fever are reported worldwide annually, although the majority of infections may be asymptomatic. Thus, *Ae. aegypti* has been the subject of populations genetics intense studies to defined genetic relationships among collections worldwide and genetic diversity within the species. *Ae. aegypti* citocrome c oxidase subunit 1 (AeCOI) is the largest of the three mitochondrial-encoded cytocrome oxidase subunits and the most conserved among 3 cytocrome oxidase coding genes mtDNA. In this study we choose *Ae. aegypti* populations representing some important commercial routes, representing 6 states of 5 demographic regions. mtDNA COI sequencings was used to investigate population structure of theses samples. Populations were obtained from 10 cities distributed in the Brazil states of Alagoas, Ceará, Mato Grosso do Sul, Paraná, Rondônia and São Paulo, from January to March 2002. The gene COI was amplified and surveyed for variation among all 104 mosquitoes. Phylogenetic analyses indicated the existence of two historical mitochondrial lineages among the six haplotypes detected among the *Ae. aegypti* populations examined. The two mitochondrial clades occurred among all collections and Santos showed high polymorphic populations with occurrence of 4 different haplotypes.

## VE13 - Molecular phylogeny of Calliphoridae using mitochondrial DNA sequences

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Forensic entomology is a discipline that mainly uses insects colleted in and around corpses to estimate the time since death or postmortem interval. Among all scavenger and neocrophagos insect groups that are related to corpes, blow flies (Diptera: Calliphoridae) are probably most important. They are also crucial for the maintenance of ecological systems, and the involvement of some species in myiasis gives them substantial medical and veteninary importance. Sequencing of the mitochondrial cytochrome oxidase I (COI) gene is particularly useful in evolutionary studies and population genetics due to the relatively high degree of variation in the region. Studies of the Calliphoridae have also focused on sequencing of the mitochondrial region encoding the COI gene. In the present study population structure of samples in Botucatu (SP) and Nova Andradina (MS) were analyzed by COI sequencing. Botucatu populations were obtained twice a month over a year from March 2003 to February 2004, in three different areas: urban, farm and wild. Traps were set from drink plastic bottles, all of them with hole on bottom. Chicken viscera were used as baits set inside the bottles. Populations of Nova Andradina (MS) were obtained in July 2004 in trash stored by the use of baits. The 310 base pairs of the mitochondrial COI haplotypes were aligned and MEGA software package was used to analyze specimens. COI haplotypes have been differentiated by 58 mutations, represented by 40 transitions and 14 transversions. The species of the genus *Chrysomya* were grouped with high bootstrap support. At species level, specimens of *C. putoria* formed a single cluster with 100% support. The specimens of *L. eximia* formed a cluster with high support. Differences from flies collect in urban and wild areas were observed and could be used in forensic studies.

## VE14 - Interaction of *Trypanosoma cruzi* with midgut proteins of *Rhodnius prolixus*

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Blood-sucking arthropods, especially insects, have been widely used in biological, biochemical, molecular, pharmacological and immunological studies. The main medical interest of these studies is due to the fact that these arthropods may be vectors for virus, bacteria, protozoan and worms that cause several important diseases. One of the strategies for controlling these diseases is based upon understanding some molecular aspects of the interaction between vectors and parasites. During their life cycles all parasites suffer the influence of a wide number of environmental molecules that interact with their surface and exert deep influence in intracellular process. These interactions and their effects will dictate the success of the infection. In the invertebrate host *Trypanosoma cruzi* binds to the surface of the insect midgut and suffer cellular differentiation. This process is crucial for the parasite life cycle, as they differentiate from epimastigote to trypomastigote, the infective form for the vertebrate hosts. Platelet-activating factor (PAF) is a phospholipid that is a potent mediator of many cellular functions in diverse biological and pathophysiological processes, including cellular differentiation, inflammation and allergy. Recently we showed that PAF triggers the process of cell differentiation of *T. cruzi* from epimastigote to trypomastigote. In the present study we observed that when the insects were artificially fed with rabbit defribinated blood with the addition of $10^{-6}$ M PAF-treated epimastigotes there was a 60% increase in the number of parasites recovered from the midgut after 10-day infection, as compared with insects fed with blood with the addition of control epimastigotes. In order to further investigate this phenomenon, we decided to determine which molecules of the triatomine midgut are involved in the interaction of the parasites with this tissue. The protein profile of *Rhodnius prolixus* midgut was obtained through SDS-PAGE. These proteins were transferred to a PVDF membrane and incubated with biotin-labeled living *T. cruzi*. The parasites bound to at least one protein of 65 kDa. Ongoing experiments aim the identification and characterization of these proteins. Supported by: CNPq, CNPq-PIBIC/UFRJ, FAPERJ, CAPES

### VE15 - Population structure of *Triatoma brasiliensis* inferred from variation in mitochondrial DNA sequences: an attempt to understanding the domiciliation, invasion and reinfestation processes

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In Brazil, interruption of the transmission of Chagas disease has been achieved through control interventions against the most important vector, *Triatoma infestans*. However, *Triatoma brasiliensis* remains as a major threat, occurring in several natural and anthropic ecotopes in 12 States, presenting various rates of infestation and natural infection. Recently, multidisciplinary studies showed that *T. brasiliensis* is a species complex composed of four forms which are distinct at species level (brasiliensis, macromelasoma, juazeiro, and melanica). One of the key questions to understanding the domiciliation process is whether anthropic ecotopes select a particular genetic profile, possibly resulting in a reduction of genetic variability. To address this question, the cytb gene from 121 field specimens *T. brasiliensis* (brasiliensis form) was sequenced and compared. These specimens were from seven municipalities of Paraíba State up to 180 Km apart. Sixteen specimens were from intradomiciliary, 71 from peridomiciliary and, 34 from natural ecotopes. Neighbor Joining Method and parsimony tree-building were used. Statistics to support the clades was based on the bootstrap method with 1000 replications. A total of 26 different haplotypes was observed. The haplotypic and the nucleotide diversity were 0.80 and 0.00688, respectively. Here we focus our results on Sao José da Lagoa Tapada Municipality. According to data from National Health Foundation this municipality displays one of the highest domiciliary infestation and reinfestation rates in the country. Fifty-four specimens were from this municipality. Of 10 bugs from intradomiciliary ecotopes, six shared one (unique) haplotype. The other four different haplotypes were shared with those from the sylvatic ecotopes (n=34) from where high haplotype diversity (0.80-95) and 18 different haplotypes were registered. Of the 10 specimens from the peridomiciliary ecotopes, seven shared one (unique) haplotype while the three others had haplotypes identical to bugs from the wild environment. We have ruled out the possibility that the individuals sharing the same haplotype were members of the same family because they were collected in different houses. These results highlight the role of the wild environment in the infestation and possibly in the reinfestaion processes, and suggest that a reduction on the genetic variability could be involved in the domiciliation of *T. brasiliensis*.

### VE16 - Genetic variability in natural populations of *Lutozomyia* intermedia(Lutz and Neiva 1912) (Diptera: Psychodidae:Phlebotominae) by RAPD-PCR

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In the State of Espirito Santo, Brazil, *L. (V.) braziliensis* is the ethiological agent of ACL, with *Lu. whitimani*, *Lu. migonei* and *Lu. intermedia* acting as the principal vector species. *Lu. intermedia* is widespread in the State being the most prevalent species in every endemic area. The principal goal of the present study was to evaluate the diversity and the genetic structure of natural population of *Lu. intermedia* from ACL endemic areas that exhibit distinct eco-epidemiologic patterns. Several mathematic models were employed to analyze the PCR-RAPD pattern of each specimen from two municipalities in Espirito Santo: Afonso Claudio and Viana, corresponding to populations that were collected from the domestic or peri-domestic environments of each area. The analyses found a level of genetic diversity in the *Lu. intermedia* populations, with moderate to high genetic differentiation among the populations studied. The moderate differentiation between the *Lu. intermedia* populations from the two areas could be explained by the geographic distance between the two localities, a hindrance to the genetic flow. The intradomestic populations were genetically different from those circulating in the peridomestic environments, in both studied areas. This result could reflect the distribution of behavior phenotypes. Therefore, our results are indicative that control efforts against *Lu. intermedia* acting only inside the houses would not be effective, at least in the studied areas, as there is a strong relationship between the populations from the intra- and peri-domestic environments, with the latter being essential to the maintenance of the population of this sandfly species. Supported by grants from FIOCRUZ, PRONEX-CNPq; and FAPERJ (BRAZIL).

### VE17 - *Aedes aegypti* in Brazil: The erradication really happened?

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*Aedes aegypti*, was introduced in Brazil in the 17th and 18th centuries from Africa, with the slaves. In the middle of

the 19th century this mosquito has infested the cities of the Asian continent. In the 1950s, after an eradication campaign in South America, *Ae. aegypti* was considered eliminated from Brazil. After this 1950s eradication, *Ae. aegypti* was recorded again in early 1960s. Geographic variation in the competence of *Ae. aegypti* to be infected and transmit YF and DEN viruses has been observed among populations of *Ae. aegypti* from Brazil and around the world. Thus it is be important to know the geographic origin of this vector. Such information would enable one to determine the potential hazard that the presence of this vector may represent in an area. Thirty-one sites were sampled from 16 states. The non-Brazilian samples were obtained by interchange with international researchers. Mosquito DNA was amplified using ND4 specific primers and sequenced. DNA sequences were aligned manually. The haplotype network was constructed using the program TCS. The maximum likelihood tree was constructed using PAUP*. Analysis of population genetic structure was carried out using AMOVA in ARLEQUIN 2000. Two major clades were found in the haplotype network. Results of phylogenetic analysis recovered the same two major clades. Bootstrap support for these two clades is strong. Results of AMOVA analysis show 83.38% of variation between these two clades ($Fct = 0.83381$, $P \leq 0.0001$). The geographical distribution showed the presence of Senegal-related haplotypes in the Northeast of Brazil. Central African, Asian and North American related haplotypes are present in the major ports of Brazil suggesting that the former lineage is a residue of the original african strain which arrived in Brazil with the slaves. The other lineage is result of more recent introductions, by world trade.

Support: FAPESP, CNPq

## VE18 - EFFECT OF NYMPHAL DENSITY IN THE DEVELOPMENT AND SURVIVAL OF *Triatoma klugi* (HETEROPTERA: REDUVIIDAE).

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Several triatomine bug species lives in colonies since this behavior affects their survival, development rate, fecundity and longevity. The aim of this study was to evaluate the influence of nymphal density on the life cycle duration and mortality of nymphs as well as the reproductive capacity of adults of *Triatoma klugi*. For that, recently emerged nymphs were grouped in different densities (30,60,90,120 and 150) in identical plastic flasks. Experiments were performed in triplicate and the groups were observed daily in order to register death and/or ecdysis. After emergence, couples were formed to verify pre-oviposition and oviposition periods and hatching rates. The nymphs mortality was higher in the first two instars, probably due manipulation, but no significantly difference between the groups was observed. Also, no correlation between the density of nymphs and the duration of the instars was observed. The adults from groups with the lowest densities presented a higher blood ingestion rate when compared with those from higher densities. The oviposition and hatching rates decreased in the group containing 150 when compared to the others. This result suggests that there was competition for food or even space in higher densities of nymphs, affecting directly the oviposition, hatching and ingestion rates of adults, but not the nymphal survival of *T. klugi* and duration of instars. Key words: *T. klugi*, nymphal density, development

## VE19 - Development of *Trypanosoma cruzi* in *Triatoma klugi* (Hemiptera, Reduviidae, Triatominae) under starving condition.

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*Trypanosoma cruzi* is transmitted trough contaminated triatomine feces. *Triatoma klugi* was originally found in rock formations and the specimens captured presented no gut contents, showing that starvation is a frequent and natural phenomenon related to this species. The aim of this work was to address the vector-parasite interaction under experimental starving condition. For that, two groups of nymphs were fed in *T. cruzi* infected mice during the first (first group) and the fourth (second group) instars. All nymphs were weekly fed in non-infected mice until the fifth instar. A total of 10 nymphs/group were dissected after 10, 20, 30, 60, 90 and 120 days of starvation, quantifying the number of parasites. Around 25 nymphs of the first group were fed in non-infected mice after 60 days of starving and 5 insects were dissected after 1, 2, 3, 5 and 10 days, having analyzed the number and morphology of the flagellates. The results showed a great heterogeneity between the samples. In the first group, the number of parasites within triatomines gut decreased up to 20 days of starving but increasing after 60 days. In the second group, an increasing number of parasites were observed up to the 30th day of starvation, decreasing significantly until the 120th day. In both groups, the proportion between the parasite forms was maintained during the whole period of study. We may conclude that the development of *T. cruzi* in the intestinal tract of *T. klugi* depends on the nutritive state of the insect, affecting both parasite morphology and development. Key words - *Triatoma klugi* - *Trypanosoma cruzi* - starvation

## VE20 - Differential Phosphoprotein Profile in *Aedes aegypti* Induced by Feeding and *Plasmodium gallinaceum* Infection

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Mosquitoes are the vectors of tropical diseases such as malaria, dengue, yellow fever and encephalitis. Many events are observed in adult females in response to a blood meal. These events include blood ingestion, formation of peritrophic matrix, induction of protease synthesis and their secretion in the mosquito midgut. Furthermore, these mosquitoes can ingest an infected blood and in case of

malaria infection, the midgut is the first organ to come in contact with the parasite. This complex set of events requires several signaling cascades, most of which are still unknown. The final target of every cell-signaling pathway involves the reversible phosphorylation of proteins. Therefore, the present study was designed to understand the mechanisms by which blood acquisition, digestion and malaria parasite infection are triggered by these cascades. Firstly, to identify the complete set of proteins either phosphorylated or dephosphorylated upon these events we investigated the phosphotyrosyne profile of adult mosquitoes emerged from pupae. We observed an increase on tyrosine phosphorylation. Secondly, we fed *Aedes aegypti* females with two different meals: one of them was artificial meal (AM) and the other was *Plasmodium gallinaceum* infected blood. After 24 hours, females were homogenized, submitted to SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blocked and incubated with rabbit primary polyclonal antibodies against phosphoserine or mouse monoclonal against phosphotyrosine and developed by ECL. Ours results show that seven days after infected blood meal, when oocyst is implanted in mosquito midgut, the tyrosine phosphorylation protein profile decrease significantly. Thirdly, we fed females with AM and then incubate the midguts with $^{32}$Pi for two hours. An autoradiography revealed that some protein bands had their phosphorylation increased. Identification of the above mentioned phosphoproteins will be conducted next through Edman Degradation and Mass Spectrometry. This strategy will provide us with new possible targets for control of malaria transmission.

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## VE21 - THE EGG HATCHING AND LARVA ESCAPING IN THE AEDES AEGIPTY.

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The Aedes aegypti is a proven vector of dengue. The mosquito development includes egg, four larval stages, pupa and adult. The larval stages occur in the water after the egg hatching. The larva of first instar has a dorsal head pine denominated egg buster related with the egg hatching. This study analyzes the egg hatching and larva escaping. The eggs were fixed and processed for scanning electron microscopy. The egg surface is constituted by a rigid cuticle responsible for egg protection against dissection and possible changes in the environment. The eggshells have are decorated structures with hexagonal arrays. Firstly, it was possible to observe a small protuberance in the exochorion. Probably, by internal movements of the first instar larvae in the mature egg ready to hatch. The egg buster starts to appear in a small hole over the egg surface. It appears that larva with synchronous movements of its head promotes open this small hole in the chorium. After while, this hole increases showing a broken line. As the egg hole increases, the larvae head appears in the egg surface. The larva movements broke the corium of the egg and progressively exposures the larvae. In a final stage, the larva has continuous movement to exit the broken egg. In the new free larvae, it was possible to observe details of the egg buster situated in the dorsal larvae, which has a conic smooth structure. This structure only is present in the first instar larva and disappears in the next larval stage. In conclusion, this study revealed the ultrastructural aspects of the larva escaping and confirmed the importance of the function of egg buster in this process. These results are important in the knowledge of the basic biological development of this important disease vector.

## VE22 - SCANNING ELECTRON MICROSCOPE STUDY OF THE DEVELOPMENTAL STAGES OF LUTZOMYIA INTERMEDIA E LUTZOMYIA WHITMANI (DIPTERA: PSYCHODIDAE) , A NATURAL VECTORS OF CUTANEOUS LEISHMANIASIS

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Lutzomyia intermedia and Lutzomyia whitmani are vectors of american cutaneous leishmaniasis in several endemic regions of Brazil. In this study, we analyzed the ultrastructural aspects of the vector immature stages using scanning electron microscopy observing the insect surfaces of the eggs, larvae stages- and pupae. Our results showed that they have some distinct characteristic although in general, they are very similar. Detailed observation of the egg exochorion showed that both L. intermedia and L. whitmani have ornamentation but they differ from each other in relation to their ridges. The L. intermedia exochorion has connected ridges on the surface distinctly from the L. whitmani, which presents unconnected ridges. In all the larvae stages of the two species, we observed similar aspects in the larvaes heads that were well individualized and sclerosised with prominent mouthparts. The abdominal segments were easily recognized for the presence of the pseudolegs, structures existent on the ventral surfaces. Smooth setae were also found in both species in the head and in each lateral side of the pseudolegs but their presence differ in L. whitmani, since they were found specifically around the first segment thoracic and in the last abdominal segment. Another type of uniform structures, denominated brush-like setae, were also found on the head and larva dorsal and lateral parts, except in the anal segment. The larvae of the

two species also showed similar aspects in the antennae with heart-like forms in the first stage, which changed to straight forms in the others. The pupa stages were recognized by the encapsulated shape of the insects and easy observation of topography of the eyes, wings, antennae of the future sand fly. These observations represent a study that should be used for the fundamental base to posterior taxonomic considerations. Financial support- CNPq, Fapemig and FIOCRUZ.

## VE23 - Ecto-Phosphatase Activity in Salivary Glands of *Rhodnius prolixus*: Modulation by *Trypanosoma rangeli*.

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Proteins phosphorylation plays a cardinal role in regulating many cellular processes in eucaryotes. Processes that are reversibly controlled by protein phosphorilation require not only protein kinase but also a protein phosphatase. Invasion into the insect vector salivary glands by some protozoans, such as *Trypanosoma rangeli*, is a necessary step for transmission and likely to be mediated by specific receptor-ligand interactions. In the present study we investigated phosphatase activity on the surface of *Rhodnius prolixus* salivary glands. Phosphatase activity decreased with the increase of the pH from 6.0 to 8.5 and the effects of different phosphatase inhibitors on the ecto-phosphatase activity were studied and showed a high inhibition with the classical acid phosphatase inhibitors as sodium vanadate (90%) and ammonium molybidate (73%) indicating a possible presence of an acid ectophosphatase on the surface of salivary glands of the insect-vector. Subsequently, the phosphatase activity detected on the salivary glands surface were employed to investigate the interaction between *T. rangeli* and the *R. prolixus* salivary glands. *In vitro* assays using short and long epimastigotes showed that short but not long (the invasion/adhesion forms) induced a in salivay glands phosphatase activity reduction. Preliminary *in vivo*experiments by hemocoel inoculation with short epimastigotes of *T. rangeli* demonstrated that the salivary glands phosphatase activity was two fold lower than the control, without infection. These findings may add some insights to studies on the interaction of *T. rangeli* with the insect-vector, *R. prolixus*.
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## VE24 - Differential susceptibility of three populations of *Triatoma brasiliensis* to a *Trypanosoma cruzi* strain

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In the Northeastern states of Brazil, *Triatoma brasiliensis* (Hemiptera, Reduviidae, Triatominae) is the most important domestic vector of *Trypanosoma cruzi*, the causative agent of Chagas disease. In the semi-arid regions of the "Brazilian caatinga", *T. brasiliensis* is widespread in sylvatic ecotopes, peridomestic habitats and forms abundant domestic colonies. The objective of the present work was to investigate the susceptibility of three *T. brasiliensis* populations (sylvatic, peridomestic and domestic) to a *T. cruzi* strain (TBRA/BR/2001/TB01) recently isolated from a sylvatic *T. brasiliensis* from Ceara State, Northeastern Brazil and characterized as *T. cruzi* group I. Fifth instar nymphs of each *T. brasiliensis* populations were fed on Swiss albino mice infected with the *T. cruzi* strain. After ingestion of the blood with infective forms of the parasite, the insects populations were dissected and their gut compartments (anterior and posterior midguts and rectum) carefully isolated in order to avoid mixture with each other. The parasite development was evaluated in all the gut compartments in each *T. brasiliensis* population, 1, 5, 10, 15, 20, 25 and 30 days after the blood meal. In the first evaluation (24h), the parasites were found only in anterior midgut, with their density similar in the three insect populations (p¡0.05). Five days after feeding, the parasites were found only in the posterior midgut and at the same concentration in all *T. brasiliensis* populations. Significant differences were found only $15^{th}$ day after feeding, when the parasite numbers were significantly higher in the sylvatic *T. brasiliensis* population. The total number of metacyclic trypomastigotes was highest in this sylvatic group after this time, suggesting an adaptation between the insect and parasite strains from this sylvatic ecotope. However, there were no differences in the ratio of metacyclics trypomastigotes to epimastigotes between the three populations. These results elucidate the importance of studies of the vector/parasite interaction, which can contribute to a better understanding of the biochemical and molecular aspects of such interaction. Supported by: Fiocruz, CNPq, FAPERJ, Volkswagen Foudation.

## VE25 - DIGESTION OF TRIACYLGLYCEROL IN Rhodnius prolixus MIDGUT: Role of Triacylglycerol Lipase

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Blood feeding is an essential part of development process for many arthopods. In most blood feeding arthopods, the midgut plays a crucial role because it is the primary tissue involved in processing and exporting products of digestion of the blood meal to the hemolymph. *Rhodnius prolixus* can take a large blood meal containing a substantial amount of lipids, mainly triacylglycerol. In insects, two models of lipolysis in the midgut lumen were proposed, the complete hydrolysis of triacylglycerol to free fatty acids and glycerol or the formation of free fatty acids and monoacylglycerol. Triacylglycerol lipases are enzymes that preferentially hydrolyse the outer ester links of triacylglycerols and act only on the water-lipid interface. Insect midgut triacylglycerol lipases were studied in few insects and only in crude preparations.

The data suggest that the enzyme preferentially releases fatty acid from the 1- and 3- positions, shows a preference for unsaturated fatty acids, and is activated by calcium ions, thus resembling the action of mammalian pancreatic lipases. Two days after blood meal, midguts were dissected from adult females and midgut luminal contents were removed. After this, midgut tissue and luminal contents were incubated with radiolabelled triacylglycerol ($^3$H-triolein) in the presence of Triton X-100 and unlabelled triolein, and amounts of the released fatty acid were determined for the lipase activity characterization. It was demonstrated that the amount of free fatty acids liberated was proportional to the amount of enzyme added, thereby indicating that the release of free fatty acids was due to the action of lipase. The lipase activity was affected by pH variation and showed optimum activity values at pH 5.0. It was inhibited by different concentrations of PMSF, and it was decreased to 10% of control at the concentration of 10mM. After feeding, triacylglycerol lipase activity increased, and the highest activity was observed at second day after blood meal, when digestion is very intense, and then gradually decreased. The study of digestion mechanism of triacylglycerol is important for the understanding of the regulation of lipid absortion in midgut of Rhodnius prolixus. Supported by CNPq and Faperj.

## VE26 - Sand fly saliva: in vivo and in vitro immune responses

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New World sand fly saliva contains an array of pharmacological and immunomodulatory components and the mechanisms by which these components modulate the host s immune response to Leishmania have not been fully elucidated. In the present study we have examined the immune response of mice against Lutzomyia intermedia saliva, the vector of L. braziliensis in Brazil. BALB/c mice were immunized with salivary gland sonicate (SGS), probed for a DTH reaction and sera and draining lymph nodes were collected for evaluation of the humoral and cellular immune responses. Sera from immunized mice reacted against the major proteins of Lu. intermedia SGS and analysis of cytokine expression showed an up regulation in both IFN gama and IL 4 upon in vitro stimulation with SGS. Histopathological analysis of the immunization site showed a mixed infiltrate of polymorphonuclear cells (neutrophils and eosinophils) whereas the challenge site exhibited a mononuclear cell infiltration comprised mainly of lymphocytes and monocytes, indicative of a DTH response. The effect of Lu. intermedia SGS on human cells was also investigated. In order to do so, human monocytes were incubated with SGS in the presence or absence of LPS. Cells and supernatants were harvested to determine cytokine and co stimulatory molecule expression. Lu. intermedia SGS was able to modulate the cytokine response of LPS stimulated monocytes as shown by a decrease in IL 10 and TNF alfa and an increase in IL 6 and IL 12p40 production. Moreover, we observed that Lu. intermedia SGS was able to enhance Leishmania infection of human monocytes and was able to modulate the cytokine production of infected cells as shown by an increase in IL 10 production. Collectively, these results indicate that sand fly components have an immunomodulatory effect on in vivo and in vitro immune responses which may have important consequences on the host parasite interplay. Financial support: NIH AI 30639, FAPESB, CNPq and PAPES FIOCRUZ.

## VE27 - Eicosanoid pathways in *Rhodnius prolixus* infected with *Enterobacter cloacae*.

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In several species of insects, immunity to pathogens depends on different cellular reactions described by some authors as : (i) recognition of non-self surfaces and phagocythosis by hemocytes; and (ii) nodule formation and encapsulation. The reactions involve hemocyte degranulation and activation of the prophenoloxidase (proPO) system, hemocyte microaggregation and the beginning of phagocytic activity mainly by plasmatocytes and later attachment of developing nodules to organs of the host.
In the present work, we investigated the effect of feeding $5^{th}$-instar larvae of *Rhodnius prolixus* on blood containing eicosanoid biosynthesis inhibitors (dexamethasone and indomethacin) on the cellular immune reactions in the hemolymph when the insect is challenged by inoculation with *Enterobacter cloacae* $\beta$12 5 days after oral treatment.
Our results demonstrated that dexamethasone and indomethacin at a dose of 50 $\mu$g/ml of blood meal did not alter biological parameters such as feeding, mortality, excretion and molting of *R. prolixus*. Hemocoelic infection of insects previously fed on blood containing dexamethasone or indomethacin, both at a dose of 50 $\mu$g/ml, increased significantly the mortality when compared with controls, as a consequence of the higher bacteria number. Also, we demonstrated a reduction of the number of hemocyte microaggregation (nodules formation) and of prophenoloxidase activity in the hemolymph. The numbers of circulating hemocytes in the hemolymph were unaltered within both experimental and control groups of insects.
Based on these results, we suggest that the eicosanoid pathway could mediate the hemocyte microaggregation reactions in the hemolymph of insects inoculated with *E. cloacae* $\beta$12 and treated with dexamethasone and indomethacin.

## VE28 - Activity of agglutination against *Trypanosoma cruzi* in extract from digestive tube of *Rhodnius prolixus*

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*Rhodnius prolixus* is a main triatomine vector of Chagas' disease. In earlier studies have been demonstrated activities in extracts from the digestive tube of *R. prolixus* that agglutinated rabbit erythrocyte and *T. cruzi* epimastigotes. These both activities are current from distinct factors present in the digestive tube of *R. prolixus*, whereas the supernatants from extracts incubated with erythrocytes, don't clumped more this kind of cell but continues aggregating the parasites. On the other hand, supernatants from extracts incubated with epimastigotes remain agglutinating only erythrocytes. In assays varying the insect diets was verified that the agglutinated activity against *T. cruzi* come from the feeding with plasma, while the hemagglutination is related with the erythrocytes present in the diets. Moreover, plasma or serum alone incubated with epimastigotes also showed an agglutinated activity. Thus, masses of aggregated parasites are formed *in vitro* even after the heat complement inactivation and this activity remained in the insect crop after blood feeding. Anion exchange chromatography followed by gel filtration and electrophoresis analysis, demonstrated that plasmatic proteins with high molecular weight are involved in epimastigote aggregation. The kinetic of parasite aggregation and the partial inhibition by p-nitrophenol suggest that hydrophobic interactions are involved in this activity.
This work was supported by grants from PACDT program, CNPq and FAPERJ. CBM is a CNPq research fellow.

## VE29 - LIPASE ACTIVITY IN THE FAT BODY OF RHODNIUS PROLIXUS AT DAYS AFTER MEAL

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The triacylglycerol is a main form of energy storage available in living organisms. It is an energy dense substance which is accumulated by several body tissues, specially adipose tissue and liver in mammalians. Utilization of stored TAG as an energy source requires its mobilization from these depots and transfer into blood plasma. TAG is mobilized by hormone sensitive lipase that releases fatty acids. A number of co-ordinated cAMP-dependent intracellular processes culminate in the phosphorylation and activation of HSL and allow the access to the intracellular lipid droplets. The study of the mechanisms for TAG mobilization for energy production in non-mammalians species, particularly insects, may have important implications for the understanding of intracellular mechanism of TAG mobilization and release. The objective of this work is the characterization of TG-lipase activity in the fat body of Rhodnius prolixus. Females, ten days after blood meal, were dissected and the fat bodies homogenized. The homogenates were centrifuged at 20,000 x g for 30 min at 4o C and the infranatant and fat cake were used as enzyme source. The enzyme was incubated with 3H-triolein in the presence of Triton X-100 and unlabelled triolein, and the amounts of released fatty acids were determined. When we increased the enzyme amount a increase on TG-lipase activity was observed until 0.4 mg of enzyme source add. This enzyme showed a optimal activity between pH 7.4 and 8.5 and PMSF was effective in inhibiting this activity. When we measured this activity on different days after blood meal, it didn't vary on second, sixth, tenth and seventeenth days after blood meal. These results suggested a mechanism similar to that observed in mammalians in which the TG-lipase activity is always present, but its effect is regulated by phosphorylation and translocation to the lipids droplets. Supported by CNPq and Faperj

## VE30 - Tyrosine phosphatase activity induced by Dengue Vírus infection in *Aedes albopictus*

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Dengue virus is classified in the family *Flaviviridae* (genus *Flavivirus*) which includes 50 other members such as West Nile, Yellow Fever and Japanese encephalitis virus. Transmitted by the mosquito *Aedes aegypti*, Dengue is the most prevalent arthropod-borne virus affecting humans, with most 50 million new cases per year. One of the most important mechanisms of signaling in eucaryotic cells is protein phosphorylation on tyrosine residues, which plays a pivotal role in development by regulating cell proliferation, differentiation and migration. Cellular phosphotyrosine levels are regulated by the antagonistic activities of the protein-tyrosine kinases and protein phosphatases. The objective of this work is to study the signaling mechanism involved in the infection by dengue virus in the vector insect. Therefore, we used a *Aedes albopictus* cell culture (C6/36) to characterized a tyrosine phosphatase activity. C6/36 cells with five days culture were homogenized in 20mM sodium acetate pH 4,5 buffer, 1mM DTT, 10 mM EDTA and a protease cocktail inhibitor. Total protein content was determined by Lowry. The phosphatase activity was measured with a synthetic substrate p-nitrophenil phosphate (pNPP) during 1 hour at 37 $^0$C and 0,02 mg total protein. This enzyme has a pH optimum in the range of 4,5 - 5,0. The reaction is linear up to 180 minutes and 4 mM pNPP is the ideal concentration. To classify this enzyme, we used several phosphatases modulators and observed that 1 mM sodium fluorite (NaF) inhibited 96% the activity, 0,1 mM ammonium molybdate inhibited 95% and 0,1mM sodium vanadate 60%. This data indicate that this enzyme correspond to a tyrosine phosphatase. When the cell culture was infected with Dengue virus type II, we observed a increase of 500% in phosphatase activity four days after in-

fection. This results indicate for the first time a mechanism where a virus manipulate the mosquito signaling pathways. Supported by AMSTMH, FAPERJ, CNPq and PADCT.

### VE31 - Apyrase, phospholipase $A_2$ and PAF-acetylhydrolase are constitutively expressed in the saliva of triatomines

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The saliva of blood-sucking insects shows many pharmacological activities that antagonize the host's hemostatic response. For example, the inhibition of platelet aggregation plays an important role during blood feeding. In accordance with this feature, we compared apyrase, phospholipase $A_2$ and platelet-activating factor (PAF)-acetylhydrolase activities in salivary gland homogenates (SGH) of *Triatoma infestans*, *Rhodnius brethesis*, *Rhodnius millesis*, *Rhodnius robustus*, *Rhodnius pictipes* and *Dipetalogaster maximus*. Apyrases are nucleoside triphosphate-diphosphohydrolases, which remove inorganic phosphate from ATP and ADP. The apyrase activity was tested with Fiske-Subbarow reagent in the presence of the following divalent ions: $Mg^{+2}$, $Cu^{+2}$, $Co^{+2}$, $Mn^{+2}$, $Zn^{+2}$ and $Ca^{+2}$ using AMP, ADP and ATP as substrates. We found apyrase activity in the presence of $Ca^{+2}$ upon ADP and ATP in all SGH tested. The maximum and minimum activities were detected in SGH of *R. millesis* and *D. maximus*, respectively. Apyrases of *T. infestans* and *D. maximus* also employ $Mg^{+2}$, $Mn^{+2}$ and $Co^{+2}$ as cofators. The PAF-acetylhydrolase (PAF-AH) activity was identified in salivary gland homogenates of these insects using the PAF-AH fluorogenic substrate 2-(6-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino) hexanoyl-1-hexadecanoyl-*sn*-glycero-3-phosphocoline in a $Ca^{+2}$-independent manner. The phospholipase $A_2$ activity was identified using $PLA_2$ substrate 2-(12-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino) dodecanoyl-1-hexadecanoyl-*sn*-glycero-3-phosphocoline in a $Ca^{+2}$-dependent manner. All homogenates exhibited activities of $PLA_2$ and PAF-AH at the same level. The enzyme $PLA_2$ plays a key role in various biological processes, including homeostasis of cellular membranes, lipid digestion, host defense and signal transduction. It could be related to innate immunity of the insect. The apyrase and PAF-AH activities in salivary gland of hematophagous insects are related to the prevention of ADP and PAF-induced platelet aggregation of the host during blood sucking, thus helping the insect to obtain its blood meal.

This research is sponsored by CNPq.

### VE32 - Lipid accumulation in the fat body of *Rhodnius prolixus*: effect of decapitation

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In insects, during digestion, exceeded lipids are transported to the fat body to be stored as triacylglycerol in lipid droplets.

The objectives of this work were to determine the variation of lipid content in fat body of *Rhodnius prolixus* during the days following a meal, to analyze the effect of decapitation on this process and to correlate these variations with morphological changes in lipid droplets. For determination of absolute mass, lipids from fat body were extracted and analyzed by TLC. For morphological measures, haematoxylin stained sections were visualized in optical microscope.

Our results showed that females have a large increase in triacylglycerol content around the $4^{th}$ day after the meal, which remain constant until the $13^{th}$ day, and then regularly decreased. In males, differently, triacylglycerol contents increased gradually until the $10^{th}$ day and then gradually decreased. We also observed that males store more lipids then females during all digestion cycle. Decapitated males have the same storage profile as normal ones. Considering these data, we can exclude the possibility of a hormonal factor from head to be involved in the storage and mobilization of lipids by fat body. By microscopic analysis, we could see a close relation between the content of triacylglycerol and the size of lipid droplets in fat body: in the $2^{nd}$ day after blood meal, when the level of triacylglycerol is low, there were few small lipid droplets. On the other hand, in the $6^{th}$ and $10^{th}$ days, when there are more lipids in the fat body, the size and abundance of lipid droplets were larger.

**Supported by: FAPERJ and CNPq**

### VE33 - Blastocrithidia culicis colonizes the insect vector Aedes aegypti: clues to understand opportunistic infections caused by monoxenous trypanosomatids in vertebrate hosts

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Monoxenous trypanosomatids comprise a group of protozoa which infects invertebrates, mainly insects belonging to Diptera and Hemiptera groups. Recently, cases of HIV-positive patients that had opportunistic infections caused by these protozoa were reported. In order to verify the possible modes of transmission of monoxenous trypanosomatids to vertebrate hosts, we investigated morphological aspects of insect vectors colonization by lower protozoa. *Blastocrithidia culicis* is a monoxenic protozoan which harbors a symbiotic bacterium in the cytoplasm. The endosymbiont supplies the protozoan with nutrients, such as hemin and vitamins.

Moreover, presence of the endosymbiont induces biochemical and morphological changes in the host trypanosomatid, affecting its surface charge and carbohydrate composition. A remarkable morphological feature of these monoxenic trypanosomatids is the lack the paraxial rod present in digenetic counterparts. Previously we showed that endosymbiont bearing trypanosomatids, specially *B. culicis*, interacted with Diptera cell lines and with gut epithelium. Prompted by these findings, we sought to investigate if *B. culicis* could cololize *Aedes aegypti* females. Here we report that *B. culicis* was capable of colonizing the insect digestive tract up to 38 days after feeding. Histological and ultrastructural analysis showed that this protozoan binds to the microvilli through their flagella. After longer periods of feeding, protozoa reached the haemocel, crossing the insect gut through the tight junctions that join adjacent epithelium cells. Interestingly, the lack of the paraxial rod in *B. culicis* does not preclude formation of adhesive contacts with the epithelium lining, thus challenging previously held notion about the functional role of this specialized structure. In summary we report that *B. culicis* are able to colonize *Aedes aegypti*, setting a precedent for studies in another insect vectors. These findings suggest that the endosymbiotic bacterium may indirectly contribute to the successful transmission of heteroxenic protozoan to vertebrate hosts, hence.

Supported by FAPERJ and CNPq

## VE34 - Expression of recombinant antibodies with anti-dengue activity in mosquitoes Aedes aegypti.

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Dengue fever (DF), caused by four distinct serotypes of dengue virus (DEN), is the most important arthropod borne viral disease in humans, being transmitted by the domestic mosquitoes Aedes aegypti and A. albopictus. Efforts to control DEN based on elimination of water containers breeding sites through insecticide use and community participation have not been totally successful due to several factors including insecticide resistance. Therefore, as an alternative strategy, transduction systems based on the double subgenomic Sindbis RNA virus (dsSIN) have been employed to express heterologous proteins and antisense RNAs in A. aegypti to test blocking molecules to parasites and virus infections in the mosquito. Recombinant monoclonal antibodies (scFv-Mab) showed to be a powerful weapon for blocking vector-borne diseases as recently, our group developed and expressed the variable portion of a Mab (N2-scFv) that prevents P.gallinaceum sporozoites invasion of mosquito salivary glands. We are currently developing different scFv-Mabs that recognize DEN in A. aegypti as a prelude to test whether it is possible to express anti-DEN-scFv-Mab in transgenic mosquitoes to block virus transmission. The Mab 1A10-2 (Henchal et al., 1985) recognizes the DEN-2 envelope protein (prE) by immunofluorescence and Western blot analyses of infected mosquito cells and shows significant neutralization activity in cultured cells. The Mabs 2H2 and 1B7 detected premembrane protein (prM) of all four serotypes by IFA and Western blot analysis. The Mab 3H5 recognizes the prE and it is a strong neutralizer of DEN-2 virus. The corresponding heavy- and light-chain variable regions encoding the anti-dengue Mabs 1A10-2, 1B7, 3H5 and 2H2, were engineered to produce the ScFv constructs 1A10-scFv, 1B7-scFv, 3H5-scFv and 2H2-scFv, and were cloned into a dsSIN expression plasmid to produce infectious virus for inoculation into DEN infected adult mosquitoes. Supported by FAPESP

## VE35 - Multiple introductions of the Yellow Fever Mosquito *Aedes (Stegomyia) aegypti* in Peru

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*Aedes* mosquitoes are found in tropical and subtropical zones throughout the world, and are responsible for the transmission of a number of viral and filarial human pathogens. The most well known member of the genus, *Aedes aegypti* (Linnaeus, 1762), is capable of transmitting viruses such as yellow fever, dengue (serotypes 1-4) and chickungunya fever, all of which can cause severe morbidity and mortality.
The phylogeographic relations of 3 *Aedes aegypti* Peruvian populations was obtained, based on genetic variability of NADH4 mtDNA fragment analysis. After sequencing of twenty-two samples 3 haplotypes were detected. All sequences from Lima and Iquitos showed the same haplotype (HI) and sequences from Piura showed two other haplotypes (HII and HIII). Haplotype HIII is separated from haplotype II by 11 mutational steps. AMOVA has shown that the major part of the detected genetic variation occurs at interpopulational level. The significant value $\Phi$st=0.609 suggests that Piura population is structured in relation to Lima and Iquitos populations and the genic flow of the NADH4 gene is restricted in Piura when compared to the two others populations. The relationship among haplotype HI and Piura's haplotype HII indicates the introduction of the same mtDNA lineage for these locations. However the existence of genetically distant HIII haplotype suggests the introduction of at least two *Ae. aegypti* distinct lineages in Peru.

Costa-da-Silva A. L. is a CNPq fellowship.

## VE36 - Is catalase an essential enzyme to the cattle tick *Boophilus microplus*? Roles on longevity and on oogenesis.

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The cattle tick *Boophilus microplus* is an ectoparasite that is exclusivelly hematophagous. It ingests large quantities

of blood in a single meal, reaching a hundred fold its own weight before eating. Digestion of hemoglobin results in high amount of heme, that must be removed to avoid oxygen radical formation. Catalase is an antioxidant enzyme capable to remove hydrogen peroxide. So, it is easy to foresee that catalase may execute a noble function in this animal.
Catalase activity has been well correlated with longevity. Catalase inhibition in *Boophilus* resulted in diminished survival and was accompanied by an increase in hydrogen peroxide level observed in digestive cell through fluorimetric analysis, using either microscopy or fluorimeter. Hemolymph eletrophoretic profile suggests that animals treated with a catalase inhibitor, show proteolytic degradation of yolk proteins. Together, these results may justify the diminished reproductive efficiency and survival observed in animals whose catalase was inhibited.
Catalase activity was more sensitive to this inhibitor in ovarian than in the midgut, if the drug was administred in the hemocoel. In this condition, rates of ovoposition and egg hatching were diminished in a dose dependent maner and were accompanied by an augmented level of hydrogen peroxide. Hemolymph of animals treated with the drug accumulate HeLp (heme-binding lipoprotein), probably as a consequence of heme not being delivered to ovaries. In conjuction, these data may explain the diminuted availability of eggs.
Therefore, we propose that catalase is an essential enzyme that allows the cattle tick to conclude its biological cycle, contributing to its survival and to its oogenesis.
Supported by: CNPq, HHMI, CAPES, PRONEX

## VE37 - Xanturenic acid in the *Aedes aegypti* midgut, its kinetics during blood digestion and its antioxidant activity

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Xanthurenic acid (XA) is a trypthophan derivative present in mosquito which was recently recognized as being the gametocyte activation factor (GAF) for the protozoan *Plasmodium*, the etiologic agent of malaria. However, virtually no studies were done toward its function on mosquito digestive physiology. In our study we show the presence of very high concentration of XA in the midgut lumen of *Aedes aegypti*. The midgut content was fractionated by HPLC using a reverse phase column and a diode array detector. XA was identified based on its light absorption and on the fragmentation pattern by mass spectrometry. The concentration of XA vary during digestion, raising to millimolar values at 24h after blood meal and coming back to low levels at 42h. The fluorescence spectrum of XA changes markedly in the presence of heme iron and calcium, three important molecules in the gut scenario in *Aedes aegypti*, indicating its binding to these species. In addition we showed that XA is capable to protect azolectin liposomes from peroxidation induced by heme or ferrous ion. Taken together these results indicates that XA is an important molecule of the digestive physiology in *Aedes aegypti* and possibly have an antioxidant role in the adult insect blood meal digestion.
Supported by CNPq, HHMI, Capes, Faperj

## VE38 - Lysophosphatidylcholine from *Rhodnius prolixus* salivary glands enhances *Trypanosoma cruzi* transmission

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*Trypanosoma cruzi* is the causative agent of Chagas' Disease. *Rhodnius prolixus* is an important vector of this disease in America. During blood-feeding saliva is injected into the host and avoids hemostasis. Meanwhile parasites are deposited in feces very close to the bite wound. Lysophospholipids have emerged in the last few years as powerful modulators of cell signaling. We have recently shown (Golodne et al., JBC 278(30): 27766-71, 2003) that *R. prolixus* saliva contains the bioactive phospholipid lysophosphatidylcholine (lyso-PC) which controls host hemostasis. When *T. cruzi* invasion occurs through the bite wound, parasites face a cell environment previously stimulated by saliva. In the present study we investigated whether *R. prolixus* saliva or its purified phospholipids (Lyso-PC and PC) were able to enhance *T. cruzi* infection towards BALB/c mice in vivo and against BALB/c peritoneal macrophages in vitro. Mass Spectrometry analysis showed salivary lyso-PC contains palmitic acid (16:0) as its fatty acid component. When *R. prolixus* saliva was added to the interaction medium, the association index of the interaction between *T.cruzi* and mouse peritoneal macrophages was enhanced by 2-6 fold. Also, total lipid extract from *R. prolixus* saliva containing equivalent amounts of lyso-PC, as well as lyso-PC itself, showed 2-3 fold increase in the association index, as compared to the control system, in the same kind of assay. In vivo infection in BALB/c mice generated an acute blood parasitemia, which were at least the double in saliva- or lyso-PC-treated mice, as compared to the control ones. Furthermore, *R. prolixus* saliva acts as a chemotractant in BALB/c pleurisy assay. The number of mononucleated cells doubled 6 to 24 hrs after injection. Also, we did not observed dramatic alterations in the cytokine expression profile as deduced by a 32-cytokine commercial protein array assay. This array indicates that saliva shows minor levels of immuno-modulation. Altogether, these results suggest that LPC from *R. prolixus* saliva, is a enhancing molecule of Chagas' Disease transmission. So far, this is the first demostration of a vector-derived molecule modulating *T. cruzi* infection.
Supported by CNPq, PADCT, FAPERJ and IFS.

## VE39 - Development and Evaluation of a Reverse Transcription Real-Time Development and Evaluation of a Reverse Transcription Real-Time PCR assay for detection and quantification of all Dengue virus serotypes.

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In spite of all scientific advances, infectious diseases are still the main cause of death in the world. Among these illnesses, one of the most important is dengue. Dengue is transmitted by mosquitoes, mainly Aedes aegypti, infecting thousands of people in the world annually. Up to now, no effective vaccines, medicines or even a fast and reliable diagnosis technique is available for this disease.

In the last decade, advances in sequencing techniques have made available the genomes of several strands of the different Dengue serotypes. Based on this information, a large number of traditional reverse transcription techniques and a small number of Reverse Transcription Real-Time PCR protocols to identify Dengue virus RNA have been proposed.

To our knowledge, no Brazilian group has ever used any of the described protocols succesfully. For this reason, we decided to develop a novel reverse transcription Real-Time PCR protocol adequated to our needs.

Initially, sequences from several strains of all four Dengue virus serotypes were retrieved from GenBank and aligned using Clustal X. The most conserved regions in the analyzed samples were identified and, a 80 bp fragment located at the 3' UTR of the virus with the appropriated characteristics for PCR (melting temperature, thermodynamic energies, G+C content, and potential for dimerization and hairpin formation) was picked and used as a template for the synthesis of the two primers and the probe for the Real-Time reaction. We decided to use the TaqMan technology with a probe labelled with 5' 6-carboxyfluorescein (FAM) and 3' 6-carboxytetramethyl-rhodamine (TAMRA). Invitrogen Superscript III one step qRT-PCR system was used for the PCR. To make the virus quantification more precise, we are using a plasmid containing the sequence used to generate the primers as a copy number standard. The optimal conditions for the PCR (primers, probe and template RNA concentrations and cycle conditions) have been determined using infected Vero and c6/36 cells as experimental samples. We are now using this methodology to investigate the efficiency of different Dengue virus serotypes of replicating in different cell lineages and primary cultures and in the mosquito Aedes aegypti as well as to identify and quantify virus amounts in human serum samples.

## VE40 - Anophelines as vectors of malaria in three riverine communities in Amapa State, Brazil.

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Introduction: Knowledge about the dynamics of malaria transmission in different epidemiological situations is important for the planning of control strategies. The rate of infection in anophelines is an important tool in this process.

Objectives: Determine which anophelines are potential vectors of malaria in three riverine communities to better understand local transmission dynamics.

Materials and Methods: Anophelines were captured from April/03 to July/04 in São Raimundo Pirativa, São João and Santo Antonio, situated along Rio Matapí, Amapá. After identifying 91,648 anophelines, they were tested by ELISA to verify the CS protein of *P. falciparum*(Pf); *P. vivax* 210, *P. vivax* VK 247; 63,328 were tested for *P. malariae*. Mosquitoes were tested in pools of up to seven mosquitoes per pool.

Results: SR *An. darlingi*: *P.f*(0.72%),*P.v.*210 (0.96%), *P.v.* 247 (0.56%) and *P.m.* 1.14%); *An. marajoara*: *P.f*(0.87%), *P.v.* 210 (0.58%), *P.v.* 247 (0.97%) and *P.m.*(0.69%); *An. nuneztovari*: *P.f*(0.39%), *P.v.*210(0.58%), *P.v.* 247 (0.58%) and *P.m.*(0.48%); *An. triannulatus*: *P.v.*210 (0.59%) and *P.v. 247* (1.18%); *An. intermedius*: *P.v.* 210 (0.59%) and *P.m.*(0.32%). SJ: *An. darlingi*: *P.f.* (0.95%), *P.v.* 210 (1.52%), *P.v.* 247 (0.63%) and *P.m.* (0.88%); *An. marajoara*: *P.f.* (0.70%), *P.v.*210 (1.0%), *P.v.*247 (0.88%) and *P.m.* (0.64%); *An. nuneztovari*: *P.f.* (1.16%); *P.v.* 210 (0.36%) and *P.m.* (0.64%); *An. triannulatus*: *P.f.* (1.22%), *P.v.*247 (0.92%) and *P.m.* (0.35%); *An. intermedius*: *P.f.* (0.38%), *P.v.* 247 (0.38%). SA: *An. darlingi*: *P.f.* (0.66%), *P.v.* 210 (1.38%), *P.v.* 247 (0.65%) and *P.m.* (1.28%); *An. marajoara*: *P.f* (1.17%), *P.v.* 210 (1.46%), *P.v.* 247 (1.22%) and *P.m.* (1.25%); *An. nuneztovari*: *P.v.*210(1.73%), *P.v.* VK 247 (0.15%) and *P.m.*(0.70%); *An. triannulatus*: *P.f.*(1.04%), *P.v. 210 (0.46%),* P.v. *247 (0.70%) and* P.m.*(0.45%);* An. intermedius*:* P.f. *(0.23%),* P.v. *210 (0.23%),* P.v. *247 (0.08%) and P.m.* (0.10%). Conclusions: *Anopheles darlingi* is considered the major malaria vector in the Amazon region; however this study shows that other species may play important roles in the transmission of malaria including *An. marajoara*, *An. intermedius*, *An. nuneztovari*, and *An. triannulatus*.

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## VE41 - *LUTZOMYIA INTERMEDIA* ( LUTZ & NEIVA, 1912) SANDS FLIES ARE SUCCESSFUL INFECTED WITH *LEISHMANIA (VIANNIA) BRAZILIENSIS* AN EXPERIMENTAL MODEL

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The aim of this study is to understand interactions between *Leishmania (V.) braziliensis* and its vector *Lutzomyia intermedia*, both from an endemic area of American Cutaneous Leishmaniasis (ACL), and also analyze the influence of feeding and refeeding with blood from possible vertebrates reservoirs. Sand flies were captured and subjected to infection with *Le. braziliensis* amastigotes and the model of development of the *Le. braziliensis* in gut of *Lu. intermedia* was demonstrated. After each artificial feeding, the guts of each sand fly were examined to observe the presence, location, morphology and density of the parasites. In the first series of experiments, flies were allowed to feed with mouse blood seeded with *Le. braziliensis* amastigotes. The infection rates were 86.5% in $1^{st}$ day and decreased to 42.9% in $10^{th}$ day after blood meal. A second set of experiments was performed in which flies were infected using different vertebrates blood. In the $5^{th}$ day, 100% of the sand flies fed with ox blood were infected, but when they were fed with donkey blood, the infection rate fall to 33.3%. In the last set of experiments, infected sand flies were allowed to re-feed in a second normal blood meal. In order to do this, the sand flies were firstly infected with mouse blood seeded with amastigotes and in the $4^{th}$ day with blood from distinct animals. In $7^{th}$ day after the initial infection, the analyzed guts of each sand fly showed an increase in the promastigote number. In all experiments, it was noted the preferential location of the promastigotes in the pyloric triangle. Additionally, the analyses of the promastigote morphology showed that they are predominantly haptomonads and nectomonads forms.

## VE42 - The Peritrophic Matrix of *Lutzomyia intermedia* Lutz & Neiva, 1912 (Diptera: Psychodidae: Phlebotominae) an American Cutaneous Leishmaniasis vector.

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In blood-feeding insects, a layer of acellular material, called peritrophic matrix (PM), is formed around the ingested blood meal and separate the food bolus from the midgut epithelium. This structure is considered as having an essential role in the physiology of the blood digestion and therefore, in the parasite-vector relationship. In a previous article, one of us demonstrated the role of the PM in the survival of *Leishmania major* inside its sand fly vector. The *L. intermedia* PM was analyzed by scanning electron microscopy (SEM) focusing on its ultrastructure and formation. This sand fly has been incriminated as a vector of American Cutaneous Leishmaniasis (ACL) in many Brazilian endemic areas. Female sand flies were allowed to feed on hamsters, the midguts were dissected at the following times after blood meal: 3, 6, 12, 24, 48 and 72 hours. Then, they were fixed and processed for SEM microscopy. Several aspects of PM ultrastructure and formation were observed. At 3 h, constitutive elements of a PM started to be observed close to the midgut epithelium. At 6 h, the PM is better visualized close to the midgut epithelium. The PM is almost formed around 12 after the blood meal. Twenty-four hours it was observed a complete formed PM. Forty-eight hours after blood feeding, the debris of the PM was observed distant from the midgut epithelium showing an evidence of digestion. At this time, most of the blood meal has been already digested. The PM was not observed after 72 hours. Our results confirm the presence of PM in *L. intermedia* and its relationship with the blood digestion. This study may serve as base for studies of vectorial competence in this specie.

Key words: peritrophic matrix, *Lutzomyia intermedia*, midgut, scanning electron microscopy
SUPPORT: CNPq, FIOCRUZ, FAPEMIG, FAPESB.

## VE43 - A NEW ARTIFICIAL FEEDING METHOD FOR EXPERIMENTAL INFECTION OF PHLEBOTOMINE SANDFLIES

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Experimental infection of phebotomine sandflies with *Leishmania* spp. has been used to study the parasite-vector relationship and the transmission dynamics of leishmaniasis. There are many experimental studies on the behavior of *Leishmania* spp. in sandflies, including the study of *Leishmania chagasi* development in *Lutzomyia longipalpis* (Lutz & Neiva, 1912), the most important vector of visceral leishmaniasis in Brazil. The classic method in the studies is the artificial membrane feeding on *Leishmania* spp. infected tissues. Direct feeding on infected animals also has been used as source of parasites, but it has failed in some cases. In this study, we describe a new method of sandfly infection: artificial membrane feeding on macrophages infected *in vitro* with *Leishmania chagasi*. Murine macrophages (cell line J774) were infected for 4 hours with stationary-phase promastigotes of *Le. chagasi*, at a ratio of 10:1 (parasites/macrophage).

Infected cells were then mixed with heparinized mouse blood and 5-6 days old sandflies were allowed to feed on it through a chick skin membrane in an artificial glass feeding device. In the fifth day following artificial blood feeding, digestive tracts of female flies were dissected and observed under a light microscope. We found 100% of infected flies in all the experiments.
KEY WORD: phebotomine sandflies, *Lutzomyia longipalpis*, *Leishmania chagasi*, Macrophage J774, *in vitro* infection.
SUPPORT: TMRC - NIH, CNPq, FAPESB.

## VE44 - Heme detoxification by the blood-sucking insect *Rhodnius prolixus*

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*Rhodnius prolixus* presents protective mechanisms against heme-induced oxidative damages generated by digestion of blood hemoglobin. One of them might be the degradation of heme itself. Previously, we demonstrated that the product of heme degradation in *R. prolixus*, located in the heart and gut, is an isomer gamma of biliverdin IX (Rp-BV) bound to two cysteine residues. This new biliverdin compound suggests a novel heme degradation pathway.

This work begins with the characterization of this new pathway. Females were injected with cysteine, heme or both. Hearts homogenates were than analyzed by reverse-phase HPLC. Four peaks with retention time longer than Rp-BV were intensified in the presence of heme, suggesting that they correspond to intermediates of heme degradation pathway. Two peaks were purified and analyzed in an Electronspray Mass Spectrometry for mass determination (ES-MS) or collision-induced fragmentation for structure identification (ESMS/MS). Species of 971 and 793Da were found, suggesting the addition of two peptides Gly-Cys to the heme molecule before its degradation into Rp-BV. The masses of the two other intermediates were identified, using a NanoHPLC coupled with an Electronspray Mass Spectrometer where heart homogenates from females injected with heme were applied. Species of 932 and 881Da were found, corresponding to a biliverdin bound to two Gly-Cys peptides and a biliverdin with one of the peptides already processed to cysteine.

In order to understand the physiological role of heme degradation in *R. prolixus* a study was also carried out with the gut. Adult females were fed with blood supplemented with SnPPIX, a heme oxygenase inhibitor. SnPPIX induced, in vivo, a decrease in the levels of apoRHBP and an increase in the lipid peroxidation levels in the hemolymph, measured by TBARS assay. Taken together, these results indicate that heme degradation is an important mechanism of defense against heme toxicity.

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## VE45 - Cathepsins characterization in *Triatoma brasiliensis* midgut

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In contrast to other hematophagous insects, triatomines digestive enzymes have been characterized as acidic proteases similar to cathepsins. These insects may have evolved from sap-sucking hemiptera, which lost their ability to produce proteases due to their feeding habits. The triatomines may have developed mechanisms to use their lysosomal proteases primarily to digest their blood meal. Cathepsins B and D activities have already been described in triatomine midgut, and recently, the cathepsin L gene was sequenced from *Rhodnius prolixus* midgut. The aim of this work is to characterize, using specific activity measurement and gene sequencing the gut cathepsins of *Triatoma brasiliensis*, the main vector of Chagas disease in Northeastern Brazil. In the present work, the specific activities of cathepsins B, L and D are presented and also the preliminary results from cathepsin D gene sequencing, isolated from *T. brasiliensis* midgut. Adult insects were dissected at different days after the blood meal, and their midguts, both lumen and wall, were used for enzymatic specific activity measurements. All three cathepsins were detected in the *T. brasiliensis* midgut and had higher activities in the wall than in the lumen. It was also observed that in both compartments cathepsin B (using BANA, N-a-benzoil-DL-arginine-b-nitroanilide as substrate) and L (using Z-Phe-Arg-pNA as substrate and in presence of urea), both cysteine proteases, had higher activities on the third day after feeding, while with cathepsin D (using hemoglobin as substrate), an aspartic protease, the maximum activity was detected on the ninth day after the blood meal. For cathepsin D gene isolation and characterization, insects were dissected nine days after feeding and mRNA extracted from the midgut. After cDNA synthesis, a PCR with degenerate primers was carried out and the products cloned and sequenced. Two different sequences were obtained with similarities to cathepsin D and aspartic-type endoprotease. Specific primers will be used for whole gene amplification and for evaluation of the gene expression using Real-Time PCR. Supported by: Fiocruz, FAPERJ, CNPq, Volkswagen Foundation.

## VE46 - Involvement of *Phytomonas serpens* proteolytic activities in the interaction with *Oncopeltus fasciatus* salivary glands

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Phytomonas species are parasites of both plants and insects and may cause diseases in plants of economical importance. Insects belonging to the Orders Diptera and Hemiptera are the vectors in the transmission of Phytomonas species. The hemipteran *Oncopeltus fasciatus* is not only the natural host for *Phytomonas elmasiani*, but it is also the host of different species of trypanosomatids in experimental infection. The invasion of the vectors salivary glands is one of the most important events in the life cycle of these parasites. Previous study from our group showed the ex vivo interaction between *Phytomonas serpens* and the external face of salivary glands of *Oncopeltus fasciatus*, by means of scan electron microscopy. Several parasites were observed invading an area of the basal lamina of the salivary glands, showing putative lesions in those. It has been suggested the involvement of proteolytic activities in the establishment of host infection by many trypanosomatids. In the present study we partially characterized and compared, by gelatin-SDS-PAGE, the extracellular and cellular proteolytic activity profiles of *Phytomonas serpens*, before and after the incubation of these parasites in the presence of *Oncopeltus fasciatus* salivary glands. In the conditions used in this study we observed that the parasites present at least 4 proteases, whose molecular mass range from 35 to 55 kDa. This profile was not altered when the parasites were incubated in the presence of the insects' salivary glands. Secreted proteases presenting molecular mass of 90 kDa and optimum activity at pH 10.0 have also been observed in the culture medium of the parasites. This last protease activity has not been observed in the interaction medium. On the other hand, a 140-kDa secreted protease presenting optimum activity at pH 5.5 was observed in this medium. Together, these results suggest the involvement of these protease activities in the interaction of *Phytomonas serpens* with the *Oncopeltus fasciatus* salivary glands. Supported by: CNPq, FAPERJ, CNPq/PIBIC-UFRJ and PRONEX (0885).