EP001 - **PLASMODIUM FALCIPARUM MEROZOITE ANTIGENS ASSOCIATED TO PROTECTION FROM NATURALLY ACQUIRED IMMUNE RESPONSE IN THE BRAZILIAN AMAZON**

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The acquisition of a protective immune response able to abrogate symptoms of *P. falciparum* is a common feature of people living under continuous exposure to malaria infection. Despite the identification of many important antigens on the merozoite and at the infected red blood cell surface, it is still unclear which antigens are involved in protection. Herein, we tested 24 different merozoite antigens for their recognition by parasitemic individuals from endemic areas.
Blood samples were collected from asymptomatic (24) and symptomatic (27) *P. falciparum*-infected patients, living at the Madeira River in the Western Brazilian Amazon, considered a low endemicity area. After an extensive survey of all different circulating sequences of merozoite-related genes in the patient-derived field isolates, corresponding recombinant peptides were expressed in E. coli as GST-fusions. These polypeptides represented members of the merozoite surface protein family (MSPs), apical membrane antigen (AMA1), erythrocyte binding antigen family (EBAs) and merozoite adhesin erythrocyte binding ligand (MAEBL). ELISA assays were then conducted using the recombinant peptides and plasmas from the same blood samples, including IgG subtype analyses. We found a very high frequency of merozoite antigen recognition, the maintenance of antibody levels over time, for most antigens, and the predominance of IgG1 and IgG3 subtypes in the humoral immune response. Asymptomatic patients presented a significantly higher prevalence of MSP5 and MSP9 recognition and more intense humoral response against MSP4, MSP5, MSP9 and EBA175. Symptomatic and asymptomatic patients also exhibited different patterns of IgG subclass distributions against a number of antigens. Finally, we conclude that in these epidemiologic settings a strong and simultaneous response against $MSP1_{19}$, MSP3, MSP4, MSP10 e EBA175 is an essential step to develop/maintain an asymptomatic profile.
Supported by:FAPESP

EP002 - **CLINICAL AND EPIDEMIOLOGICAL ASPECTS THAT INFLUENCE THE EVOLUTION TO CURE OR DEATH OF HUMAN VISCERAL LEISHMANIASIS IN GOVERNADOR VALADARES, MG, BRAZIL**

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Visceral leishmaniasis (LV) is one of the six endemic diseases with priority in the world. It has gained great importance, in special with its urbanization, as well as its lethality. The clinical evolution is diverse, assuming serious and lethal forms. Governador Valadares (GV) reported the first case in 2008 and non clinical and epidemiological factors were identified previously. This descriptive study analyzed demographic, clinical, laboratory and epidemiological variables from LV patients living in GV that evolved to cure or death in 2008-2010 period. The results showed that all cases were from urban zone and 76.5% male sex (p= 0, 032) and majority in the age ranging between 0 - 10 years in both sex and between 41 - 50 only for male. Regarding the diagnosis as control measured, 44.4% of the individuals looked for the health services up to 2 weeks of beginning the symptoms, and only 30.3% had diagnosis in the first attendance. The cases showed symptoms and clinical signals characteristic of LV. The treatment of choice was Glucantime® . 88,5% of them had evolved to cure. The male sex (71%) presented greater adverse reactions than the feminine. There were eleven deaths in this period, being 72.7% male and 27.3% female. Was identified co-morbidity in 72.7% (n = 8) of obits, four had co-morbidities associated with hypertension and diabetes. We observed correlation (p = 0.051) between age and comorbidity and presence of higher score in age groups over 20 years. The presence of hemorrhagic signs was the principal complication, being present in 10 of 11 deaths. We concluded that clinical and epidemiological characteristics pointed male sex as a more susceptible group and that the delay in diagnosis, comorbidities associated with the use of Glucantime® and presence of complications are possible risk factors relevant to poor prognosis of the disease.
Supported by:FAPEMIG

EP003 - **EPIDEMIOLOGICAL ASPECTS OF HUMAN VISCERAL LEISHMANIASIS IN GOVERNADOR VALADARES, MINAS GERAIS, BRAZIL**

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In Brazil, visceral leishmaniasis (VL) suffered an urbanization process during the last years. Governador Valadares (GV) reported its first case in 2008 and it is important to know what are the risk factors for the population. Between 2008 to 2010, we conducted a descriptive study in 61 patients with VL, analyzing sociodemographic, economic and epidemiological variables, such as gender, age, residence, occupation, income, presence of infected dogs, use of insecticides, sanitary conditions, and questions about attitude, knowledge and practice of VL. All cases were autochtonous with a higher number of male individuals in the age range of 0-9 years (53.84%) in the urban district of Altinópolis. Most of the individuals showed with low education (61.53%), the occupation was mostly menial jobs (46, 15%), family income 1.2 minimum wages (46.15%), and poor housing conditions, with only 3 to 5 rooms. Most infected patients had the habit of staying indoors from 18 to 22 p.m. (75%). We also investigated 27 individuals living together with VL patients. Most of them slept in the same room (83.33%) and their age range, associated with higher risk of infection was from 20-65 years (35.71%). There was frequent garbage collection in the neighborhood and most houses had backyards with elevated humidity (63.63%) and shading (72.72%). Cleaning of backyards was reported three times per week (41.66%), presence of reservoir dogs in 72.70%, and the use of insecticide in 50% of the cases. With regard to attitudes, knowledge and practices of VL, most of the infected patients (66,66%) did not know how to prevent the disease and were unfamiliar with the asymptomatic form of VL (75.00%). Only one third of the households would like to have assistance in spraying insecticide inside their home (30.70%). Some favorable factors for the expansion of VL were identified in Governador Valadares, such as male sex, age between 0-9 years, poor hygiene, large number of livestock, and inadequate housing or sanitary conditions, related to low socioeconomic status.

Supported by:Fapemig

EP004 - **LEISHMANIA AMAZONENSIS AND LEISHMANIA BRAZILIENSIS IN DIDELPHIS ALBIVENTRIS AND MICOUREUS PARAGUAYANUS OF BRAZIL**

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Leishmaniasis is kept in nature by the participation of several animal species. The aim of the study was to evaluate the presence of Leishmania spp. in skin samples of free-ranging marsupials Micoureus paraguayanus (n=95) and Didelphis albiventris (n=191), captured in Morro do Diabo State Park and in sections of its surrounding forest, in the region of Pontal do Paranapanema, São Paulo, Brazil. The samples were tested for the presence of kDNA of Leishmania spp. by PCR and by real time PCR (qPCR). All samples from D. albiventris tested by PCR were negative for the presence of kDNA of Leishmania spp. However, when tested by qPCR, the positivity was 1.6%. A positivity of 7.4% by PCR and 11.6% by qPCR was observed for M. paraguayanus and 64% (09/14) of positive animals were limited to the same forest fragment. In search of a methodology to differentiate Leishmania species, it were tested several techniques like RFLP-PCR (restriction fragment length polymorphism) for kDNA; RFLP-PCR for SSU rDNA, nested and semi-nested SSU rDNA followed by cloning and sequencing, semi-nested PCR with glucose-6-P dehydrogenase (G6PD) and Melting temperature analysis in qPCR. Success in the differentiation was achieved only in 42.8% (06/14) of the samples and being identified as L. (Leishmania) amazonensis (02/06) and L. (Viannia) braziliensis (04/06) was detected only in M. paraguayanus skin samples. While D. albiventris is the most studied marsupial species due to its urban habits, other species such as M. paraguayanus can be potential reservoirs of Leishmania spp. The high positivity observed in M. paraguayanus in the same forest fragment could suggest that these animals are restrict to this area and deforestation imbalance the ecosystem improving the dissemination of the disease. To complete the epidemiology of Leishmania, it was observed the present of the vector in the same area, in the same year. It was the first report of the presence of Leishmania kDNA in M. paraguayanus.

Supported by:Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP n°2008/07216-0)

EP005 - **SOROREACTIVITY AND CORRELATION BETWEEN IGG ISOTYPE AND CLINICAL MANIFESTATIONS IN THE CANINE VISCERAL LEISHMANIASIS**

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In Brazil, visceral leishmaniasis is a zoonosis caused by Leishmania infantum chagasi. The dog is the main domestic reservoir, being the most important link between the parasite and the man. Nowadays the Northeast region of the country has reported the majority number of cases (47,5%), followed by the Southeast (19,2%), Northern (17,4%), Middle-West (7,4%) and South region (0,2%). In State of Rio Grande do Norte, Natal and Mossoró are classified as intense transmission areas (mean of ≥ 4,4 cases in the last three years), with the lethality rates arising in during the years. In this study we performed a serological and epidemiological profile of 398 dogs belonged of 12 rural areas in Mossoró-RN. The positive results were determined by two serological tests (IFI and ELISA). All rural areas presented seropositive dogs to visceral Leishmaniasis, however, Mulunguzinho and Lorena presented very high prevalence (49% and 41%, respectively) of positive dogs. Later, 11 animals were clinically evaluated and classified in asymptomatic (n=6) and symptomatic (n=5). Onycogriphosis, weight loss, localized alopecia, furfuraceous dermatitis, skin lesions and mucosal paleness were the most pronounced symptoms. Symptomatic animals presented higher levels of IgG_{total}, IgG_1 and IgG_2 compared to asymptomatic dogs. Moreover, a positive correlation was observed between the number of clinical symptoms manifested by the dogs and the IgG_{total} ($r = 0,58$; $P < 0,05$), IgG_1 ($r = 0,65$; $P < 0,05$) and IgG_2 ($r = 0,66$; $P < 0,05$) levels detected in the serum samples. Those results indicate that the more number of lesions the dogs present, higher levels of IgG and its specific isotipes are detected in the serum. Our results demonstrate the high prevalence of canine visceral Leishmaniasis in the rural zone of Mossoró – RN.

Supported by:CNPq

EP006 - **EPIDEMIOLOGICAL EVALUATION OF HUMAN VISCERAL LEISHMANIASIS IN BARCARENA MUNICIPALITY (PA, BRAZIL) FROM 2000 TO 2009**

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Visceral leishmaniasis (VL) has a wide distribution, and it takes place in 47 countries distributed in different global regions: Asia, Europe, Middle East, Africa and Americans which is also known as American Visceral Leishmaniasis (AVL), American Kala-azar or Neo tropical Kala-azar. In Brazil visceral leishmaniasis has been increased mainly in North region. The Municipality of Barcarena (PA) is a VL endemic area where cases has been increasing in the last few years. The aim of this study was to evaluate the reported infections in Bacarena municipality from 2000 to 2009. The VL occurrence data were obtained from Secretaria de Estado de Saúde Pública do Para (SESPA) and Sistema Nacional de Agravos de Notificação(SINAN). It was registered 342 cases of visceral leishmaniasis with a reduction of cases since 2005. Visceral leishmaniasis occurred in 20 localities of Barcarena. The highest incidence was registered in the neighborhood of Vila do Conde, which is an urban area; however, in some rural localities as Cafezal and Trevo do Peteca were registered high incidence of AVL, 34 and 26 cases respectively. Most cases of visceral leishmaniasis notified comprehend the age group of 1 to 11 years old (54,97%) followed by the group of 18 to 29 years old (14,33%). According to distribution of the disease, men were the most infect group suggesting an occupational and behavioral characteristic of visceral leishmaniasis in the municipality. The results showed the importance of further studies in VL endemic areas in order to control the spread of that disease.

EP007 - **HISTOPATHOLOGICAL EVALUATION OF MICE INOCULATED WITH TRYPANOSOMA CRUZI ISOLATES FROM THE STATES OF AMAZONAS AND PARANA**

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Chagas disease has been recognized as an emerging and neglected illness in the Amazon region with hundreds of reported cases in recent decades. Pathogenicity for mice and tropism for different organs and tissues of *Trypanosoma cruzi* strains could vary according to their geographical origin. Our goal was to perform the histopathological evaluation of mice inoculated with isolates of *T. cruzi* from the states of Amazonas (AM) and Paraná (PR), obtained from different hosts. We used six isolates from AM and two from PR that were inoculated via IP (inoculum of 10,000 blood trypomastigotes/animal of each isolate) in mice, males, 18 to 20 days. Thirty days after inoculation the animals were sacrificed and fragments from heart, skeletal muscle, liver, spleen, brain, smooth muscles, and gastrointestinal tract (rectum) were obtained for making slides stained with hematoxylin/eosin and analyzed by light microscope. The tissue parasitism was recorded only for the BS48 isolate, obtained from chronic patient of Colorado (PR), the amastigotes nests were observed in heart and skeletal muscle, and intense and diffuse inflammatory process were observed in 4/8 evaluated organs: heart, skeletal muscle, liver, and spleen. The focal and diffuse inflammation, were also observed in organs/tissues of all animals inoculated with the other isolates. Intense and focal inflammatory process were observed in heart, liver and rectum of mice inoculated with AM57 isolate obtained from a the triatomine bug *Rhodnius robustus* of Apuí (AM). This same pattern of inflammation was observed in the spleen of animals inoculated with the PR1226 isolate obtained from chronic patient of Maringá (PR). The remaining animals showed focal or diffuse inflammation of the spleen, ranging from mild to severe. Smooth muscle was the tissue that presented less inflammatory processes. The *T. cruzi* isolates obtained from chronic patients in PR were more pathogenic to mice than isolates from acute patients and triatomine of AM.
Supported by:FUNDAÇÃO ARAUCÁRIA/CNPQ/UNIVERSIDADE ESTADUAL DO OESTE DO PARANÁ

EP008 - **EVOLUTION OF INFECTION IN MICE INOCULATED INTRAGASTRICALLY WITH TRYPANOSOMA CRUZI I AND II**

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The intragastric transmission of *Trypanosoma cruzi* to human and mammals occurs and more than 700 cases of acute Chagas disease were reported in Brazil in recent years, most linked to oral transmission. The purpose of this study was to evaluate the evolution of infection with *T. cruzi* I and II by intragastric (IG) route in mice. Groups of 20 male Swiss mice, 21-28 days were inoculated after 6 hours of fasting, with a volume of 0.1 mL inoculum (i) of Y (TcII) and Colombian (TcI) strains. Ten received 1 X 104 blood trypomastigotes (BT)/animal, 5 IG by gavage and 5 intraperitoneal (IP). The other 10 animals were inoculated with 5 X 104 BT/animal: 5 IG and 5 IP. Infectivity (%INF) was determined based on the results of fresh blood examination, hemoculture, and PCR. We also evaluated the mean prepatent period (PPP), the mean period of patency (PP), the mean peak parasitemia (Pmax), and the mortality rate (% MOR). For TcII, the %INF ranged from 60% (i <) to 80% (i >) by via IG and was 100% via IP. The PPP was 5 days (i <) and 3 days (i >) for the IG and 3 days for the IP group, regardless of the inoculum. The PP ranged from 12-15 days (i < IG) and 14-15 days (i > IG), and 11-12 days (IP). The Pmax ranged from 1.8-2.2 x 105 (i < IG) and 1.5-5.9 x 105 BT/0.1 mL (i > IG) and 6.3-6.8 x 105 (i < IP) and 8.3-8.8 x 105 BT/0.1 mL (i > IP). The %MOR ranged from 60 to 80% (IG) and were 100% (IP). For TcI, the %INF ranged from 0% (i < IG) to 20% (i > IG) and was 100% via IP. The PPP was 20 days (IG) and four days (IP). The PP was 22 days (IG) and ranged from 18 (i < IP) to 23 days (i > IP). The Pmax was 8.7 x 104 BT/0.1 mL (IG) and ranged from 9.1 x 105 (i < IP) to 1.5 x 106 BT/0.1 mL (i > IP). The %MOR was 0 (IG) and 100% (IP), regardless of the inoculum. Considering the parameters analyzed we conclude that the evolution of infection in mice inoculated with *T. cruzi* by IG route was less virulent compared to IP route, regardless of the *T. cruzi* DTU (Discrete Typing Unit).
Supported by:Fundação Araucária e CNPq

EP009 - **PARASITE ORGAN-SPECIFIC TROPISM IN SILVATIC RODENTS HOSTS DURING INFECTION BY LEISHMANIA (VIANNIA) BRAZILIENSIS**

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For over 30 years the primary reservoir of *Leishmania (Viannia) braziliensis* was not known, but the use of PCR to detect parasites in samples obtained from silvatic animals implicated some vertebrate species such as rodents and marsupials as primary reservoirs of *L. (V.) braziliensis* (Brandão-Filho *et al.*, 2003). Aiming to observe the infection tropism in these natural hosts, animals from established colonies of *Nectomys squamipes*, *Bolomys lasiurus* and *Rattus rattus* were experimentally infected with 10^6 promastigotes of *L. (V.) braziliensis*. After six weeks of infection, biopsies of skin, spleen and liver were processed to purify template DNA for PCR assays, using DNeasy Blood & Tissue kit (Qiagen), according to manufacturer's protocol. The detection protocol consisted in a nested PCR assay using two pairs of SSU rDNA (Small Subunit Ribosomal gene) derived oligonucleotides (Zampieri *et al.*, 2001). The first PCR used as primers (S4-S12) that amplifies a conserved SSU region in all trypanosomatids and the second reaction used primers (S17-S18) that amplifies a common region *Leishmania* genus. The preliminary results indicated a preferential hepatic tropism of *L. (V.) braziliensis* in *B. lasiurus* and *R. rattus* and a splenic tropism in *N. squamipes*. The G6PD (Glucose-6-phosphate dehydrogenase) based real time PCR assay (Castilho *et al.*, 2008) is now being performed to quantify and determine a profile of infection tropism in these natural hosts with more sensitivity. The knowledge of pathogen-host physiology and interactions will be important to improve measures of control.

Supported by:FAPESP, FACEPE, CNPq

EP010 - **ANTI-LEISHMANIA IGG4 AS A BIOMARKER OF CURE IN DIFFUSE CUTANEOUS LEISHMANIASIS PATIENTS**

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Diffuse Cutaneous Leishmaniasis (DCL) is a rare form of cutaneous disease, characterized by the presence of multiple chronic nodular skin lesions containing many parasites. Patients do not develop specific cell mediated immune response and are resistant to treatment. It has been shown that Leishmania HSP83 antigen and IgG4 subclass antibody response can be useful in the diagnosis of active DCL. The aim of this study was to evaluate if it is possible, through total immunoglobulin G and subclasse profiles to predict cure in DCL patients. To do so, ELISA was performed using soluble leishmania antigen (SLA), recombinant Leishmania HSP70 and HSP83 and sera from active DCL (n=12), active visceral leishmaniasis(VL) (n=15) and active cutaneous leishmaniasis (CL) (n=13). We also employed sera from cured DCL patients, identified after clinical follow-up, without active lesions for the last four years and conversion of the Leishmania skin test.. We detected different IgG and subclass responses to SLA, HSP70 and HSP83, with sera from all clinical forms. A high production of IgG4 subclass for DCL patients' sera was observed when compared with VL and CL patients´sera in which predominated IgG2 and IgG1 subclass, respectively. In patients with active DCL, patients have high levels of IgG4 (OD: 0.7396) against all antigens tested, however, this effect was marked for HSP83. After cure, sera reactivity to HSP83 decreases significantly (OD:0.2279). Ours results show that the different antibody subclass response in active and cured DCL patients, using recombinant Leishmania HSP83, raises the possibility of using of this antigen and IgG4 subclass ELISA as a biomarker of cure in DCL patients.

Supported by:CNPq, FIOCRUZ, CYTED.

EP011 - **OCCURRENCE OF THE INTESTINAL AMOEBIASIS IN INDIGENOUS TAPIRAPÉ COMMUNITIES LIVING IN THE AMAZONIAN REGION OF MATO GROSSO STATE, BRAZIL.**

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The indigenous Indian population of Mato Grosso State is presently around 23,000 and includes the ethnic Tapirapé, who inhabit the Amazon region of the state. The Tapirapé live in two Indigenous land reserves: Karajá in Santa Terezinha, MT and Tapi'itãwa, located 30 km from the city of Confresa, MT. This locality is populated by some 542 Indians who live in six villages. We know that the frequency of intestinal parasites, especially intestinal amoebae, is very high amongst indigenous communities. Because of this we decided to study the prevalence of intestinal amoebae in fecal samples of all the Indian villages Tapirapé TI Tapi'itãwa. Collection vials were distributed to the entire community with the help of indigenous health agents. A total 362 samples were collected during the months of July and August 2008. They were examined using Hoffmann and Ritchie techniques in a laboratory that we established in Confresa. Of the total samples analyzed Endolimax nana was the most prevalent parasite with 249 (69%) followed by Entamoeba coli with 242 (67%), E. histolytica/dispar with 134 (37%) and Iodamoeba butschlii with 31 (9%). Of the total samples analyzed, 51 (14%) were diarrheal stools and although the association of E. histolytica/dispar with diarrheal diseases was not significant ($p>0.05$) it was present in 16 (31%) of total diarrheal stools examined. However there was a significant association ($p<0,001$) of E. coli and E. nana with diarrhea. The data corroborates the vast majority of studies performed in Amerindian villages that report elevated prevalences of amoebae. More studies are needed to understand the etiology of the gastroenteric syndromes afflicting the Tapirapé but it is likely that E. histolytica/dispar is responsible for some of the cases of diarrhea. It is hoped that these kinds of studies will contribute to improving the health of indigenous people in Brazil and Latin America.
Supported by:CNPq, DECIT, FAPEMAT, FUNASA