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Brazil pertaining to the States of Pará and Rondônia. These same sera were also tested in their ability to recognize two recombinant proteins representing two merozoite surface antigens of *P. vivax*: the merozoite surface protein 1 (MSP1) and the apical membrane antigen 1 (AMA-1). Anti-VIR antibodies were detected against each recombinant protein tested and yet the prevalence of such antibodies was significantly lower than the prevalence of antibodies against MSP1 and AMA1. Studies are now in progress to evaluate the presence of cross-reactive epitopes among the antigens encoded by the different *vir* sub-families using sera of mice immunized with each recombinant protein.

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IM81 - COMPARISON OF THE REACTIVITY BETWEEN ANTIGENS OF *LEISHMANIA (L.) CHAGASI, L.(L.) AMAZONENSIS* AND *LEISHMANIA SP.* (BIO-MANGUINHOS) IN THE SERO-DIAGNOSIS OF VISCERAL LEISHMANIASIS BY THE INDIRECT IMMUNOFLUORESCENCEC TEST.

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Introduction: antigenic specificity still represents a controversial point of discussion with regards the standardization of an antigen for use in the serodiagnosis of human visceral leishmaniasis (HVL) by the indirect immnofluorescence antibody test (IFAT). For this reason we have sought to compare the reactivity between antigens of *L.(L.) chagasi* (amastigotes and promastigotes), *L.(L.) amazonensis* (amastigotes) and a *Leishmania sp.* (promastigotes) from Bio-Manguinhos. Objective: to standardise an antigen for the diagnosis of HVL by the IFAT. Material and methods: *Sera*: 90 serum samples from patients with a previous serological diagnosis of HVL were randomly selected, together with 30 samples from individuals resident in Belém, Pará, with no previous evidence of infectious.Antigens were prepared from the promastigotes of *L.(L.) chagasi* (strain MCAO/BR/1998/M18011, Imperatriz, Maranhão State), amastigotes of *L.(L.) amazonensis* (strain IFLA/BR/1966/PH8, Belém, Pará), and promastigotes of the *Leishmania sp.* from Bio-Manguinhos. The antigens of amastigotes were made on IFAT slides by dab-smears of pieces of liver, spleen and skin of hamsters infected with the respective parasites. The promastigote antigen of *L.(L.) chagasi* was prepared from stationary phase cultures in Difco B45 medium, with a suspension of 3 x 10^6 parasites/ml. The 3 antigens were distributed on IFAT slides, fixed with acetone, and preserved at -20 °C. The Bio-Manguinhos antigen was used following the maker's instructions. *Serological test*: the IFAT was carried out using anti-IgG (Bio-Manguinhos) for the 4 antigens, with positive sera considered to be those with a titre equal or above 80. *Statistical analysis*: we used the screening-test and curve ROC (IC 95%, of the programme Bio-Estat 2.0) to evaluate the sensibility and specificity, and the Dunnett (ANOVA) ($p < 0,01$) to evaluate differences between the averages of the antigen titres. Results: the amastigote antigen of *L.(L.) chagasi* atained a 100% sensibility and specificity level. That of *L.(L.) amazonensis* amastigotes achieved a 87% sensibility efficiency and a 93% specificity efficiency. The *Leishmania sp.* (Bio-Manguinhos) antigen gave sensibility and specificity efficiencies of 88% and 90%, respectively. ROC curve values were d=0,00 for amastigotes of *L.(L.) chagasi*; d=0,17 for promastigotes of the same parasite; d=0,15 for amastigotes of *L.(L.) amazonensis*; and d=0,16 for promastigotes of the *Leishmania sp.* (Bio-Manguinhos). With regards differences between averages of the reacting sera, it may be noted that the amastigote antigen of *L.(L.) chagasi* (6.366) was significantly ($p < 0,01$) better than the promastigote antigen of the same parasite (3.712); that of *Leishmania sp.* Bio-Manguinhos was 1.299; and that of amastigotes of *L.(L.) amazonensis* 1.070. Conclusion: the results show that the *L.(L.) chagasi* amastigote antigen is the antigen of choice for the sero-diagnosis of HVL and for monitoring chemotherapy of this disease.

IM82 - EVALUATION OF IGG SUBCLASSES ANTI-*LEISHMANIA* BY ELISA IN DOGS WITH AMERICAN VISCERAL LEISHMANIASIS (AVL) IN RIO DE JANEIRO/RJ.

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Introduction: The dog is the main reservoir of the AVL in urban areas. High levels of IgG are detected in canine AVL, and also in asyntomatic animals with high degree of parasitism in the health skin and viscerals. Deplazes et al. (1995), suggest that the titles of IgG1 and IgG2 are safer indicators for the status of the illness than the IgG. In this work we investigate, through ELISA, the seroprevalence of IgG and subclasses IgG1 and IgG2 anti-*Leishmania* in dogs of AVL endemic area, evaluating their importance for the diagnosis of the illness. Methodology: It was used in ELISA a partially soluble antigen of promastigotas forms of *L. (L.) chagasi*. For this study the samples of serum had been classified in the following groups: group I - 20 serum of dogs with positive parasitologic diagnosis (8 of symptomatic dogs and 12 of asyntomatics); group II -16 dogs without parasitologic diagnosis, with positive serologic; group III- 3 dogs with negative parasitologic and group IV - control group of healthy animals. Results: In group I the seroprevalence in ELISA for the asyntomatics animals was 8.3% (1/12) for IgG1 and 100% (12/12) for IgG2 and for the symptomatic animals was 12,5%(1/8) and 100% (8/8) respectively; in group II the seroprevalence for IgG1 was 43,7% (7/16) and 100% (16/16) for IgG2; in groups I and II the seroprevalence for IgG was 100%; in groups III and IV all the sera had been not reactors for IgG and its subclasses. Conclusions: The IgG2 was prevalent and detected in high levels in dogs with AVL, however, in these same animals, IgG1 was detected in low levels. On the contrary to many authors, significant difference was not observed between the levels of IgG1 and IgG2 when correlating these subclasses with the presence or absence of clinical signals. The sensibility and specificity of ELISA for IgG2 detection were higher than IgG detection, and the agreement with the indirect immunofluorescense, seems to strengthen the safe use of this test for the diagnosis of the canine AVL.

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IM83 - IMMUNE RESPONSES AND PROTECTION INDUCED BY A COMBINED LACK AND *MYCOBACTERIUM* HSP65 DNA VACCINE AGAINST *LEISHMANIA (L.) MAJOR* INFECTION

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The LACK (*Leishmania* homolog of receptors for activated C kinase) antigen is a 36 KDa protein highly conserved and expressed in promastigote and amastigote forms of *Leishmania*. Immunization of BALB/c mice with a truncated (24-kDa) version of LACK, protein or DNA, confers strong protection against *L. (L.) major* infection. Here, we compared the protective effect of na encapsulated LACK DNA vaccine against *L. (L.) major* and *L. (L.) amazonensis* infection in BALB/c mice. Development of Th1 immune responses are essential for protection against *Leishmania* infection. *Mycobacterium* HSP65 shares high homology with *Leishmania* HSP proteins and is able to induce high levels of IFN-γ, when tested as a DNA vaccine against *Mycobacterium* infection. Thus, *M. leprae*