CYTED-RIMLEV

WORKSHOP ON CANINE VISCERAL LEISHMANIASIS

CPqGM-FIOCRUZ (SALVADOR, BA, BRAZIL)

MARCH, 28-30, 2012

Development of a more sensitive *L. infantum* recombinant protein-based chromatographic test for the rapid serodiagnosis of early-infected dogs.

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Visceral leishmaniasis is currently a major public health problem, being the dog the main reservoir in urban areas. In Brazil, the disease control is based on the diagnosis and euthanasia of all *Leishmania infantum* (syn *chagasi*)-infected dogs showing seropositivity to infection. The diagnostic test recommended by the Brazilian Ministry of Health to identify the infection in dogs is based on indirect immunofluorescence method. However, this technique showed low sensitivity and is time-consuming leading to mistakes on diagnosis and, subsequently, many infected animals are not removed or are removed lately from the endemic areas. This scenario favors the maintenance of a source of infection in these areas. Serological tests based on the whole parasite or its extracts, showed a high rate of false-positive results due to cross reactivity with other parasites. In sum, the establishment of a serodiagnostic test with better sensitivity and specificity, as well as, a greater speed of execution than those who are currently in use, is an important task to achieve. This project aims to develop a rapid test for serodiagnosis of canine visceral leishmaniasis, that is fast, simple, specific, sensitive and inexpensive and that will be applied to the Public Health network. Tests using multiple antigens are more sensitive since different dogs, when infected with *Leishmania*, hardly show the same immune response to *Leishmania* infection and produce antibodies against the same set of antigens. Thirteen recombinant antigens were previously selected using a genomic and a cDNA library of *L. infantum*. Selected fragments were produced using *Escherichia coli* (BL21(DE3)pLysS) and were transformed with plasmid constructions in pRSET with a histidine tag. The recombinant antigens were purified by affinity chromatography. A serum panel was constructed including sera obtained from 79 naturally infected dogs originary from an endemic area, 132 negative dogs from non-endemic and endemic areas, as well as dogs infected with other canine infection, including chrlichiosis (45), babesiosis (11) and tegumentary leishmaniasis (20). The reactivity of 90 sera against seven (Lci1A, Lci2B, Lci 6, Lci 7, Lci 8, Lci 10 and Lci 12) out of the 13 *L. infantum* antigens was evaluated, using a based multi-antigen print

imunoassay (MAPIA) method. Two (Lci1A, Lci2B) out of these six antigens evaluated showed promising results and were standardized and tested in the Dual Path Plataform (DPP). Lci1A and Lci2B were individually impregnated in separated membranes and tested against the serum panel. These proteins showed different degrees of sensibility and specificity related to culture result, for Lci1A 63% of sensitivity and 90% of specificity and for Lci2B 79% and 90%. When both antigens were impregnated in the same membrane, the sensitivity rose to 88% and specificity remained 90%. The evaluation of these antigens with a larger number of sera is now being performed. These results show that some of the antigens have good reactivity to sera from dogs infected with *Leishmania*. These two antigens, Lci1A and Lci2B are promising candidates to compose a rapid test for diagnosis of canine visceral leishmaniasis. After the evaluation and selection of more 2 or 3 antigens, we approach develop a rapid test based on a multiple antigen DPP.