IMPROVEMENT OF DIAGNOSIS OF CANINE VISCERAL LEISHMANIASIS (CVL) BY INDIRECT ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) USING NEW RECOMBINANT ANTIGEN

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Canine visceral leishmaniasis (CVL) is focus of many studies, because dog are important reservoirs of *Leishmania* parasite. In Brazil, euthanasia of infected dogs is one of the methods used to control the disease. The diagnosis is ELISA and IFA based (MS, 2006). However, these techniques have limitations, requiring the development and improvement of diagnosis methods. Our group has published a new recombinant antigen of *L. infantum chagasi*, potentially applicable in diagnosis of CVL (Desouza et al., 2013). This work aims to improve the ELISA diagnosis using this antigen. Immunoassays were performed varying the form of antigen purification (manual or FPLC), antigen amounts (0.1-0.5 µg), serum dilutions of positive and negative dogs (1:40, 1:80 and 1: 160) and substrates system OPD and TMB. We evaluated the influence of antigen conformation using it in native or denatured condition. In the native state protein was diluted at different concentrations in refolding buffer for 24 and 48 hours and evaluated for enzymatic activity. In the denatured state, the protein was kept in 8M urea. The best results were 0.1 µg antigen and serum dilution 1:160 for purified antigen in the FPLC. TMB and OPD did not present significant differences. Manual purification increased OD both in negative and positive sera indicating the necessity to use lower amounts of antigen and higher dilution of sera. Besides, protein diluted 40 and 50 times with refolding buffer 24 hours presented the best enzyme activity. However, ELISA diagnosis using the refolded protein was not more effective than denatured protein. Our data confirms potential of this antigen for diagnosis of CVL, demonstrating that it is possible to improve detection of antibodies levels varying test conditions. The best conditions will be used in standard serum libraries, comparing data and developing rapid diagnosis for immunochromatography.

Key words: diagnosis, ELISA, leishmaniasis.

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