TITLE: EVALUATION OF A *LEISHMANIA INFANTUM* SECRETED METALLOPEPTIDASE AS A SEROLOGICAL MARKER FOR LEISHMANIASIS.

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ABSTRACT

Leishmaniasis are cosmopolitan and anthroponotic diseases for which are estimated 350 million humans in risk zones for disease transmission. Such high number of potentially threatned people prompts the application of sensible and specific diagnostic methods for adequate treatment of humans and dogs, that can also be affected by these diseases. The traditional leishmaniasis diagnostics are based on the detection of anti-*Leishmania* antibodies and the gold standard for diagnosis is the visualization of amastigote forms in biological samples. However, the introduction of dog vaccination in recent years generated the need for new serological markers which can have potential to differentiate between vaccinated and infected dogs. Many antigens with potential to be used as diagnostic markers for Leishmaniasis are found in secretome studies of *Leishmania* spp., making these studies useful for the discovery of novel markers. Since these molecules are frequently exposed to the host immune system they may elicit stronger antibody responses. The objective of this study was to evaluate a secreted metalopeptidase of *L.infantum* as a diagnostic marker for leishmaniasis. The *in silico* analysis of the protein revealed possible B cell epitopes in the protein sequence. The identity between this protein and proteins of other Leishmania species was higher than 85% and lower than 18% when compared with proteins of *Canis familiaris* and *Homo sapiens*. The coding gene for this protein was amplified by PCR and the amplicons of desired size were ligated into pGEM-tEASY. These plasmids were then purified and sequenced. A clone without mutations in the gene was selected and was sub-cloned into the pET-28a expression plasmid, followed by tranformation in *Escherichia coli* Rosetta strain. The bacteria were then induced to produce the protein, which was purified by affinity chromatography and tested in ELISA assays agains sera from infected and healthy dogs. The results in ELISA assay with sera dilutions of 1:50 and 1:100 showed high sensitivity and specificity to differentiate between infected and non-infected dogs. These data give credibility to the hypothesis of usage of this protein as a possible diagnostic marker for leishmaniasis. Tests to confirm the results of sensitivity, specificity of the assay are under way as well as, sera from human patients and vaccinated dogs will be evaluated soon.

Keywords: *Leishmania*, leishmaniasis, metallo-peptidase, heterologous protein, diagnosis

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