Prodution of a recombinant multiepitope protein for Canine Visceral Leishmaniasis Diagnosis.

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Introduction: Canine visceral leishmaniasis (CVL) is a public health problem, being classified as a neglected disease. CVL is caused by the protozoan *Leishmania infantum* species and transmitted by the sandfly, being domestic dogs the main reservoir of this disease. Several kits based on enzyme immunoassays (EIA) are available in the market to detect anti-Leishmania antibodies.However, these kits frequently do not present a good performance, showing low sensibility and specificity. In order to circuvent this problem, the multiepitope strategy is a promisse alternative. **Objective:** The present work aims to develop a recombinant protein containing conserved epitopes to be used in the CVL diagnosis. **Material and methods:** In this work, a recombinant multiepitope protein (rMELEISH) was developed. It was possible based on conserved and immunodominant epitopes from, alpha-tubulin, kinesin 39, heat shock protein 70 and heat shock protein 83.1. This synthetic gene was cloned into pET21a vector. The recombinant protein was purified using a Ni-NTA chromatography, and ELISA experiments were performed in which reactive and non-reactive canine serum samples, were used. **Results and discussion**: This synthetic gene was cloned and sequenced prior to expression. *Escherichia coli* BL21 (λDE3) was transformed with the resulting plasmid. A bacterial clone was induced with 1mM IPTG and 2,5h after induction, the protein with an apparent molecular mass of ~25 kDa was obtained. The expression and purification were successful, as well as the tests performed for the construction of an “in house” ELISA based on rMELEISH, being able to distinguish positive and negative canine sera samples for leishmaniasis. The results showed that 35ng of protein were enough to sensitize the plate (Sarstedt). The ELISA assays showed that this protein may be used for the development of a kit with diagnostic purposes. **Conclusions:** The rMELEISH is a promising alternative for the Leishmaniasis diagnosis, with potential to develop an inexpensive diagnostic test with high degree of specificity.

Key words: Leishmaniasis, Canine Visceral Leishmaniasis, diagnosis, recombinant multiepitope protein.
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