Serodiagnosis of canine visceral leishmaniasis using a *Leishmania*-specific hypothetical protein and its non-described specific B cell conformational epitope

Daniela P. Lage[1]; Vívian T. Martins[1]; Mariana C. Duarte[1,2]; Lourena E. Costa[1]; Laura M. Dimer[3]; Amanda C. S. Kursancew[3]; Miguel A. Chávez-Fumagalli[1]; Danielle F. de Magalhães-Soares[4]; Beatriz C.S. Salles[1]; Thaís T.O. Santos[1]; Daniel S. Dias[1]; Patrícia A.F. Ribeiro[1]; Fernanda F. Ramos[1]; Mariana P. Lima[1]; Grasiele S.V. Tavares[1]; Débora V.C. Mendonça[1]; Danielle Vale[2]; Daniel Menezes-Souza[1,2]; Bruno M. Roatt[2]; Ricardo A. Machado-de-Ávila[3]; Eduardo A. F. Coelho[1,2]

[1]Programa de Pós-Graduação em Ciências da Saúde: Infectologia e Medicina Tropical, Faculdade de Medicina and [2]Departamento de Patologia Clínica, COLTEC, UFMG, Brazil. [3]Programa de Pós-Graduação em Ciências da Saúde, Universidade do Extremo Sul Catarinense, Criciúma, Brazil. [4]Departamento de Medicina Veterinária Preventiva, Escola de Veterinária, UFMG, Brazil.

The serodiagnosis of canine visceral leishmaniasis (CVL) presents problems related to its sensitivity and/or specificity. In the present study, a new *Leishmania*-specific hypothetical protein, LiHyD, was produced as a recombinant protein (rLiHyD) and evaluated in ELISA experiments for the CVL serodiagnosis. LiHyD was characterized as antigenic in a recent immunoproteomic search performed with *Leishmania infantum* proteins and the sera of dogs developing visceral leishmaniasis (VL). Aiming to compare the efficacy between whole proteins and synthetic peptides, two linear and one conformational B cell epitopes of LiHyD were synthesized and also evaluated as diagnostic markers. The four antigens were recognized by the sera of dogs suffering VL. On the contrary, low reactivity was observed when they were assayed with sera from non-infected healthy dogs living in endemic or non-endemic areas of leishmaniasis. In addition, no reactivity was found against them using sera from dogs experimentally infected by *Trypanosoma cruzi*, *Babesia canis*, or *Ehrlichia canis*, or sera from animals vaccinated with the Leish-Tec® vaccine, a prophylactic preparation commercially available for CVL prevention in Brazil. As comparative diagnostic tools, a recombinant version of the amastigote-specific A2 protein and a soluble crude *Leishmania* extract were studied. Both antigens presented lower sensitivity and/or specificity values than the LiHyD-based products. The rLiHyD presented better results for the CVL serodiagnosis than its linear epitopes, although the peptide recreating the conformational epitope resulted also appropriate as a diagnostic marker of CVL. To the best of our knowledge, this is the first study showing the use of a conformational epitope derived from a *Leishmania* protein for serodiagnosis of CVL.

Keywords: Canine visceral leishmaniasis; conformational epitope; ELISA; hypothetical proteins; *Leishmania*; serodiagnosis.

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Evaluation of a vaccine composed of a new *Leishmania*-specific hypothetical protein in the protection against visceral leishmaniasis

Daniela P. Lage[1]; Vívian T. Martins[1]; Mariana C. Duarte[1,2]; Miguel A. Chávez-Fumagalli[1]; Beatriz C.S. Salles[1]; Thaís T.O. Santos[1]; Daniel S. Dias[1]; Patrícia A.F. Ribeiro[1]; Fernanda F. Ramos[1]; Mariana P. Lima[1]; Grasiele S.V. Tavares[1]; Débora V.C. Mendonça[1]; Daniel Menezes-Souza[1,2]; Bruno M. Roatt[2] ; Eduardo A. F. Coelho[1,2]

[1]Programa de Pós-Graduação em Ciências da Saúde: Infectologia e Medicina Tropical, Faculdade de Medicina and [2]Departamento de Patologia Clínica, COLTEC, UFMG, Brazil. [3]Programa de Pós-Graduação em Ciências da Saúde, Universidade do Extremo Sul Catarinense, Criciúma, Brazil. [4]Departamento de Medicina Veterinária Preventiva, Escola de Veterinária, UFMG, Brazil.

In this work, the effect of vaccination of a newly described Leishmania infantum antigenic protein has been studied in BALB/c mice infected with this parasite species. The LiHyD protein was characterized after a proteomic screening performed with the sera from dogs suffering visceral leishmaniasis (VL). Its recombinant version was expressed, purified and administered to BALB/c mice in combination with saponin. As a result of vaccination and ten weeks after challenge using a infective dose of L. infantum stationary promastigotes, vaccinated mice showed lower parasite burdens in different organs (liver, spleen, bone marrow and footpads´ draining lymph nodes) than mice inoculated with the adjuvant alone or the vaccine diluent. Protected mice showed anti-Leishmania IgG2a antibodies and a predominant IL-12 driven IFN-γ production (mainly produced by CD4+ T cells) against parasite proteins whereas unprotected controls showed anti-Leishmania IgG1 antibodies and parasite mediated IL-4 and IL-10 responses. Vaccinated mice showed an anti-LiHyD IgG2a humoral response and their spleen cells were able to secrete LiHyD specific IFN-γ, IL-12 and GM-CSF cytokines before and after infection. The protection was correlated to the Leishmania-specific production on nitric oxide. Altogether, the results indicate that the new LiHyD protein could be considered in vaccine formulations against VL.

Keywords: Visceral leishmaniasis; vaccine; LiHyD; *Leishmania*, immune response.

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