**Specific serodiagnosis of canine visceral leishmaniasis applying antigenic mimotopes selected by a subtractive phage display technology in *Leishmania infantum***

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Visceral leishmaniasis (VL) is a zoonotic disease that is endemic to Brazil, where dogs are the main domestic parasite reservoirs, and the percentages of infected dogs living in regions where canine VL (CVL) is endemic have ranged from 10% to 62%. The serodiagnosis of CVL, however, is hampered by various factors, mainly due to the variable sensitivity and/or specificity values obtained with the different antigens employed, leading to the occurrence of a large number of false-positive results caused by cross-reactivity with other organisms, such as *Trypanosoma cruzi* and *Trypanosoma caninum*, *Leishmania braziliensis*, and *Ehrlichia canis*. Additionally, there are two commercially-available Brazilian vaccines (Leish-Tec® and Leishmune®) used to prevent the CVL that can induce the production of high levels of *Leishmania*-specific antibodies in vaccinated animals, causing them to be diagnosed as infected animals in distinct serological trials. The present study describes a sequential subtractive selection through phage display technology from polyclonal antibodies of negative and positive sera that resulted in the identification of potential bacteriophage-fused peptides that were highly sensitive and specific to antibodies of HVL. Initially, a negative selection process was performed, in that phage clones were adhered to purified IgGs from healthy and *T. cruzi*-infected dogs to eliminate cross-reactive phages. The remaining supernatant non-adhered phages were submitted to positive selection against IgG from the blood serum of dogs that were infected with *Leishmania infantum*. Phage clones that adhered to purified IgGs from the CVL-infected serum samples were selected, identified and employed in the serological analysis. Eighteen clones were identified and their reactivity was tested by a phage-ELISA against the serum samples from infected dogs (n=31) compared to those from vaccinated dogs (n=21), experimentally infected dogs with cross-reactive parasites (n=23), and healthy controls (n=17). Eight clones presented sensitivity, specificity, and positive and negative predictive values of 100%, and they showed no cross-reactivity with *T. cruzi*- or *E. canis*-infected dogs, as well as with sera of animals vaccinated with Leish-Tec® or Leishmune®. Our study identified eight mimotopes of *L. infantum* antigens with 100% accuracy for CVL serodiagnosis. The use of these mimotopes by phage-ELISA proved to be an excellent assay that was reproducible, simple, fast, and inexpensive, and it can be applied in CVL-monitoring programs.

**Keywords**: Visceral leishmaniasis; phage display; mimotopes; serodiagnosis; ELISA.

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