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Performance of *Leishmania* PFR1 recombinant antigen in serological diagnosis of asymptomatic canine leishmaniosis by ELISA

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Abstract

Background: *Leishmania infantum* is a protozoan parasite transmitted by phlebotomine sand flies that causes life-threatening disease in humans and dogs. The dog is the primary reservoir of the parasite and early diagnosis of canine leishmaniosis is crucial at the clinical and epidemiological level. The currently available serological tests for CanL diagnostic show limitations therefore the aim of the present study was to investigate the diagnostic performance of an indirect antibody ELISA based on the *Leishmania infantum* recombinant antigen PFR1 in asymptomatically infected dogs. One hundred fifty-six dogs including *Leishmania*-free experimental Beagles and pet dogs from England, Scotland and Leishmania-endemic Murcia in Spain, were tested with the assay. The later were also tested with two commercial *L. infantum* crude antigen ELISAs (INgezim and Civtest, respectively) and a real-time kinetoplast PCR test.

Results: Anti-PFR1 antibodies were detected in the four groups of dogs, and the mean log-transformed optical density (OD) values were lowest in Beagles and in dogs from England and highest among dogs from Murcia ($p < 0.05$). Using the highest OD in beagles as the PFR1 ELISA cut-off point, the estimated seroprevalence was 27% (14-40%) in dogs from Murcia, 4% (0-9%) in dogs from Scotland and 3% (0-8%) in dogs from England ($p < 0.05$). Seroprevalence in dogs from Murcia according to the INgezim and Civtest ELISAs were 24% (12-37%) and 31% (18-45%), respectively, whilst the prevalence of infection based on PCR in these dogs was 73% (60-86). The percentages of PFR1-positive dogs that tested negative on the INgezim and Civtest ELISAs were 30% and 35%, respectively, and all of them tested positive on the PCR test. Relative to the PCR, the specificity, sensitivity and area under the ROC curve of the PFR1 ELISA were 100%, 36% and 0.74 (0.63-0.86), respectively.

Conclusions: The ability shown by the PFR1 ELISA to detect infected dogs that go undetected by the crude antigen ELISAs is clinically and epidemiologically useful and PFR1 could be considered a candidate for a multi-antigen-based immunoassay for early detection of *L. infantum* infected dogs.

Keywords: Canine, Leishmania, PFR1 recombinant antigen, Serological, Diagnosis

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