

Leish085- Using qPCR for diagnosis of canine visceral leishmaniasis and quantification of *Leishmania* sp in different tissues of naturally infected dogs

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Because infected dogs are considered to be the main domestic reservoir for *Leishmania infantum* (syn *L. chagasi*) in Brazil, the diagnosis of canine visceral leishmaniasis must be made both accurately and promptly. Serologic techniques are very sensitive but present risk of cross-reaction with other diseases. First, the authors standardized a previously described qPCR protocol, targeting the kinetoplast DNA of the parasite. Using this protocol, the authors aimed to determine which tissue confers the most accurate detection of parasite DNA. In a serological surveillance in the municipality of Jequié, an endemic area in Bahia, Brazil, forty-six dogs were randomly selected and classified according to the number of clinical signs of canine visceral leishmaniasis. Dogs with one to three signs were considered oligosymptomatic, and those that presented more than three signs were considered polysymptomatic. All dogs were euthanized and splenic and blood aspirates, as well as lymph node fragments were obtained during necropsies. Aspirates and tissue samples were immediately frozen and stored at -80°C until use. ELISA and parasite culture of spleen aspirates were performed to confirm parasite infection. For each qPCR reaction, a serial dilution containing DNA from *L. infantum* in concentrations varying from 10⁵ to 10⁻² parasites was used to generate a standard curve for gene expression quantification. Each gene's expression values were normalized against the respective value of the eukaryotic 18S rRNA constitutive gene of host tissue and parasite loads were expressed as the number of parasites per of 18S rRNA gene. A ROC curve was generated to determine the positivity limit of the test. Differences between parasite loads of each tissue from oligo and polysymptomatic dogs were evaluated using Friedman test ($p < 0.05$). Using qPCR, all the 46 dogs showed positivity for the presence of parasite DNA, considering at least one of the tissues evaluated. ELISA was positive in 78% (36/46), and culture in 30% (14/46) of the dogs. Regarding the comparison of tissue analyzed, parasite DNA was highly detected in splenic aspirates, which showed positivity in 45 out of the 46 samples (97.5%, $p < 0.05$). Positivity in qPCR was detected in 78% (36/46) of blood samples, and 50% (23/46) of lymph node fragments. Using qPCR, parasite DNA was better detected in splenic aspirates in comparison with the other tissues in both polysymptomatic ($p < 0,0001$) and oligosymptomatic ($p < 0,0001$) dogs. In conclusion, splenic aspirates related to other tissues analyzed showed to be the most sensitive tissue for the detection of parasite DNA using qPCR. In our experience, spleen aspirate procedure is a well-tolerated technique, even in the most severely affected dogs. Finally, the authors recommend the use of this dog tissue to a more accurate detection of *Leishmania* infection. **Support by** FAPESB, INCT-CNPq, PDTIS, PST Veras' grant (CNPq:306672/2008-1). **E-mail:** pveras@bahia.fiocruz.br

Leish086- **Polymerase chain reaction as method of**