



CYTED-RIMLEV

**WORKSHOP ON
CANINE VISCERAL
LEISHMANIASIS**

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Using molecular detection and quantification methods to diagnose canine visceral leishmaniasis.

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Because infected dogs are widely considered to be the main domestic reservoir for *Leishmania infantum* (syn *L. chagasi*) parasites in Brazil, the diagnosis of canine visceral leishmaniasis must be made both accurately and promptly. We have previously standardized cPCR (conventional Polymerase Chain Reaction) protocol to detect the presence of *Leishmania* DNA in 45 dog spleen fragments. In addition, quantitative PCR (qPCR) technique targeting the SSU rRNA gene was used to confirm the presence of *Leishmania* DNA in the same canine spleen samples. A comparison was made between the efficacies of these molecular diagnostic techniques and conventional parasitological and serological methods. Despite the fact that the qPCR protocol provides a highly accurate quantification of parasites when targeting the SSU rRNA gene, this technique does not significantly improve the detection of parasites in spleen fragments when compared with the performance of the cPCR protocol that focused on the kinetoplast minicircle. Next, the authors standardized another sensitive qPCR protocol, targeting the kinetoplast DNA (kDNA) of the parasite, previously described by Francino *et al.* (2006) and using this qPCR protocol, the authors aimed to determine in which of the infected tissues analyzed (spleen, blood, bone marrow and lymph nodes), the detection of the parasite DNA by PCR shown to be more accurate. First, the authors determined by qPCR the parasitic load in the different tissues of infected dogs from Jequié, an endemic area located in Bahia-Brazil. Fifty-one dogs were randomly selected and were classified according to the number of clinical signs of canine visceral leishmaniasis (alopecia, weight loss, cutaneous and ocular lesions, onicogriphosis and lymphadenopathy). Dogs with one to three signs were considered oligosymptomatic, and those that presented more than three signs were considered polysymptomatic. All seropositive dogs by ELISA were euthanized and splenic and blood aspirates, as well as lymph node and bone marrow fragments were obtained during necropsies. Aspirates and tissue samples were immediately frozen and stored at -80°C until use. Parasite culture of spleen aspirates was performed to confirm parasite infection. DNA was extracted from all tissue samples. DNA samples were

then aliquoted and used to perform qPCR for detection and quantification of *Leishmania* DNA. For each qPCR reaction, a serial dilution containing DNA from *L. infantum* in concentrations varying from 10^5 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 tissues and parasitic loads were expressed as the number of parasites per concentration (ng) of 18S rRNA gene. A ROC curve was generated to determine the positivity limit of the test and differences between parasitic 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 parasitic DNA, considering at least one of the tissues evaluated. ELISA was positive in 78.3% (36/46), and culture in 30.4% (14/46) of the dogs. Regarding the comparison of tissue analyzed, parasitic DNA was highly detected in splenic aspirates, which showed positivity in 45 out of the 46 samples (97.8%, $p < 0.05$). Positivity in qPCR was detected in 78.3% (36/46) of blood samples, 50% (23/46) of lymph node samples and 44% (11/25) of the bone marrow samples. Using qPCR, parasitic DNA was better detected in splenic aspirates in comparison with the other dog tissues in both polysymptomatic ($p < 0,0001$) and oligosymptomatic ($p < 0,0001$) dogs. In conclusion, splenic aspirates related to the other tissues analyzed showed to be the most sensitive tissue for the detection of parasitic DNA using qPCR targeting kDNA. Since in our experience, spleen aspirate procedure has been well tolerated, even in the most severely affected dogs, we recommend the use of this tissue to a more accurate detection of *Leishmania* infection.