Parasites & Vectors

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Canine leishmaniasis: the key points for qPCR result interpretation

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Abstract

Background: Diagnosis and follow up of CanL is difficult since the range of clinical signs is varied and seroprevalence is high in endemic areas. The aims of this study were: i) demonstrate the advantages of *Leishmania* qPCR to diagnose and control CanL and highlight its prognostic value and ii) propose guidelines for tissue selection and infection monitoring.

Findings: This study included 710 dogs living in an endemic area of leishmaniasis. Forty percent (285/710) exhibited clinical signs consistent with CanL. Infection was detected in 36.3% (258/710) of the dogs of which 4.5% (32/710) were detected by qPCR, 16.2% (115/710) detected by ELISA and 15.6% (111/710) tested positive for both tests. Only 17.9% (127/710) of the dogs were classified sick (affected) with CanL.
All symptomatic dogs with medium or high ELISA titers were qPCR-positive in blood samples. All dogs with inconclusive or low ELISA results with high or medium qPCR parasitemia values developed the disease. Seventy one percent of asymptomatic ELISA-positive dogs confirmed by qPCR (medium to high parasitemia) developed the disease. Bone marrow or lymph node aspirate should be selected to ensure the absence of the parasite in asymptomatic dogs: 100-1,000 parasites/ml in bone marrow are detectable in blood, whereas lower parasite loads are usually negative. Almost 10% of negative samples in blood were positive in conjunctival swabs.

Conclusions: Because qPCR allows parasite quantification, it is an effective tool to confirm a diagnosis of CanL in (i) cases of inconclusive ELISA results, (ii) when the dog has not yet seroconverted, or (iii) for treatment monitoring.

Findings

Leishmaniasis is one of the main zoonosis worldwide and in some countries it is a reason of concern for public health. Canine leishmaniasis (CanL) is of great importance in veterinary medicine since dogs are believed to be the main reservoir of this parasite for humans [1]. It is endemic along the Mediterranean basin, parts of east Africa, India, Central and South America and the incidence of infection is currently spreading to non endemic areas towards the north of Europe [2] and recently emerging in North America [3]. In addition, other species have come to be infected, such as cats [4], and horses [5]. Wild canids are competent reservoirs of *Leishmania* [6], increasing the risks for humans to acquire the disease in endemic areas. Therefore, there has been a great interest in the development of new diagnostic tests.

Diagnosis of CanL is fairly difficult, since dogs manifest a very varied range of clinical signs. In CanL, infection does not equal to having the clinical disease due to a high prevalence of subclinical infections [7,8]. Moreover, it is specially challenging in endemic areas where seroprevalence rates are high [7]. Epidemiological studies in endemic zones of CanL, by means of molecular techniques, have shown that the prevalence of infection in the canine population by *Leishmania* is considerably higher than seroprevalence [8]. There are several diagnostic tests for CanL, but the correct interpretations of these are of great importance to make an accurate diagnosis of the disease [9]. Therefore, the aims of this study were: i) to demonstrate the advantages of the quantitative PCR of *Leishmania* (qPCR) to diagnose and control the disease and highlight its prognostic value and ii) propose a guideline for the tissue of choice to be analyzed in each case, as well as a guideline for monitoring the disease.

The study included 710 dogs from the LUPA Project (7 PM; subWP canine leishmaniasis). The LUPA project

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