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LeishVet Symposium – Diagnosis & Treatment of Canine Leishmaniosis: Consensus Statement Presentation

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Consensus on Diagnosis

Canine leishmaniosis (CanL) is a disease in which infection does not equal clinical illness, due to the high prevalence of subclinical infection (1). Reliable diagnostic tests are essential for the detection of Leishmania infection in dogs with or without clinical signs and/or clinicopathological abnormalities (2).

Diagnosis is usually sought for two main reasons: (i) to confirm disease in clinically suspected dogs; or (ii) to investigate the presence of infection for epidemiological studies, for screening clinically healthy dogs living in endemic regions as usually requested by the owners, to prevent transmission from healthy carriers by blood transfusion, to avoid importation of infected dogs to countries where leishmaniosis is not endemic, and to monitor response to treatment. For these reasons, it is important to separate Leishmania infection from disease and to apply different diagnostic techniques accordingly (3).

The diagnosis of CanL is complex as the clinical spectrum and clinicopathological abnormalities are both wide and not specific. Accurate diagnosis of CanL often requires an integrated approach consisting of clinicopathological diagnosis and specific laboratory tests. Several specific diagnostic techniques have been developed to facilitate the diagnosis. It is essential to understand the basis of each diagnostic test and its limitations and appropriate interpretation. Reliable and specific diagnostic tests are essential for the detection of Leishmania infection in sick dogs although they lack 100% sensitivity and specificity (3).

The most useful approaches for investigating infection both in sick and clinically healthy dogs include: (i) detection of anti-Leishmania antibodies by quantitative serological techniques; and (ii) demonstration of leishmanial DNA in tissues by applying molecular techniques (3).

In dogs with clinical signs and/or clinicopathological abnormalities consistent with leishmaniosis, the diagnostic methods also include the detection of amastigotes in stained cytological smears of aspirates from cutaneous lesions, lymph nodes, bone marrow and spleen (4, 5). Search for amastigotes by cytology could be unrewarding due to the low to moderate numbers of detectable parasites present even in dogs with full-blown clinical disease (6). Histopathology of skin biopsies or other organs complemented with the use of immunohistochemistry is useful in increasing the sensitivity of detection when a low parasite load is present (2, 6).

The serological diagnosis of CanL can be made by means of the immunofluorescence antibody test (IFAT), the enzyme-linked immunosorbent assay (ELISA) or other quantitative techniques. High antibody levels (IgG) are associated with high parasitism and disease (7). Some dogs remain seronegative for variable periods after being infected with Leishmania (8). However, due to the relatively low incubation period, sick dogs are likely to be seropositive (9). It is important to submit samples to a laboratory that runs quantitative serology and can provide an endpoint titer (IFAT) or optical density reading (ELISA) and a classification of the levels of antibodies (negative, doubtful, low, medium and high positive levels). A high level of antibodies is conclusive of a diagnosis of CanL. However, the presence of low antibody levels is not necessarily indicative of the disease and further work-up is necessary to confirm or exclude clinical leishmaniosis by other diagnostic methods such as cytology, histopathology and PCR (2, 3).

The IFAT, ELISA and qualitative immunochromatographic devices are the most commonly available techniques for detection of antileishmanial antibodies (2, 4, 10). IFAT, which uses whole promastigotes antigen, is highly specific and sensitive for the detection of clinical CanL, but may lack sensitivity to detect clinically healthy but infected dogs (11, 12). ELISA sensitivity and specificity greatly depend on the antigens employed, which mainly include soluble promastigote extracts and recombinant or purified proteins (2). Whole-parasite extracts are sensitive for the detection of subclinical or clinical canine infections but provide a somewhat lower specificity (11, 12). On the other hand, ELISA with recombinant peptides is very specific but may lack sensitivity for the detection of clinically healthy infected dogs, depending on the antigen employed (11, 13). Immunochromatography-based assays are easy to use and provide qualitative results on the spot. These kits usually have good specificity but their sensitivity is variable and their performance is still not optimal (11). False positive results due to serological cross-reactivity with other pathogens have been...
The polymerase chain reaction (PCR) has greatly improved the sensitivity of parasitological diagnosis of Leishmania infection in dogs. Different assays targeting genomic or kinetoplast DNA (kDNA) have been developed for CanL. Currently, three PCR techniques are available: conventional PCR, nested-PCR and real-time PCR. Assays based on kDNA appear to be the most sensitive for direct detection in infected tissues (2, 10, 15). PCR can be performed on DNA extracted from several tissues, blood, biological fluids or even from histopathologic specimens. PCR on bone marrow, lymph node, spleen or skin is most sensitive and specific for the detection of CanL (10, 16). PCR on whole blood, buffy coat, and urine is less sensitive than on the aforementioned tissues (16, 17). Sampling using non-invasive conjunctival swabs has proven to be very sensitive and specific for the detection of L. infantum in groups of seropositive dogs with clinical leishmaniosis (8, 18). PCR on aspirates of lymph node and bone marrow has shown to be more sensitive than microscopic detection of amastigotes in stained smears or parasite culture (6). Quantitative kDNA real-time PCR is an advanced technique that can detect extremely low parasitic loads (19) and, therefore, is more sensitive than conventional PCR (2). Real-time PCR allows the quantification of Leishmania loads in tissues of infected dogs which is important for diagnosis as well as for follow-up during the treatment of CanL (16, 20).

It is important to highlight that information provided by PCR should not be separated from the data obtained from clinicopathological and serological evaluations. These should all be combined together for a comprehensive assessment (3).

The presence of Leishmania DNA in the blood or other tissues of clinically healthy dogs living in endemic areas indicates that these dogs harbour infection (21), but they may never develop clinical disease (9). The interpretation of PCR results should be done cautiously in clinically healthy dogs and with consideration of the diagnostic procedure’s purpose. For instance, for the purpose of identifying infected dogs and preventing their importation to non-endemic areas where infection might spread via local sandfly vectors, or for the purpose of preventing transmission of infection via blood products from infected donors, PCR would be an appropriate technique in combination with quantitative serological tests (3). Confirmation of a negative status could require repeated testing after three months (22).

References


