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Diagnosis of *Leishmania infantum* in dogs and Humans: towards a One-Health Approach.

Simona Gabrielli*

Department of Public Health and Infectious diseases, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185, Rome, Italy

***Corresponding Author:** Simona Gabrielli, Department of Public Health and Infectious diseases, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185, Rome, Italy.

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Leishmanioses are globally spread vector-borne diseases caused by *Leishmania* parasites, which are transmitted by sand flies to several animal hosts, including humans. Canine Leishmanioses is a neglected zoonotic disease mainly distributed in developing countries, thought the disease is expanding throughout Europe and Americas. In the Mediterranean basin, *Leishmania infantum* and *L.major* are the zoonotic species while *L. donovani* and *L. tropica* because anthroponotic forms. Dogs represent the principal reservoir of *L. infantum*, despite other domestic and wild animal species (e.g., cats, foxes, hares) have been found infected, potentially playing an epidemiological role also because they fall outside the current control plans.

Generally, the diagnosis of Leishmanioses is made by combining anamnestic information with clinical manifestations and laboratory diagnosis. As there is currently no single "gold standard" test, the use of multiple diagnostic tests is recommended. Immunofluorescence antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA) are amongst the most common serological techniques used for the diagnosis and for clinical or research studies. IFAT method is still considered the reference technique by the World Organization for Animal Health (O.I.E. Leishmanioses 2016). However, this technique depends on the operator's skills and experience for the microscopical reading of IFAT antigen slides [1]. Moreover, appropriate setting of cut-off level is crucial in determining sensitivity (Se) and specificity (Sp) of this test. Conversely, reading of ELISA plates is rapidly operated in a plate reader at the required absorbance and, in addition to the selected cut-off, Sp and Se strongly depend on the antigen used. Western blot (WB) analysis, mainly a qualitative serological method, distinguishes the molecular weight of the *Leishmania* antigens stimulating antibody production, but is less frequently used in veterinary practice [2].

In the last decade the polymerase chain reaction (PCR) has been successfully introduced and proved to be a sensitive and powerful tool to detect *Leishmania* directly in clinical samples as well as for parasite characterization. To achieve high sensitivity, multi-copy sequences were chosen as targets for amplification, including kinetoplast minicircle sequences (about 104 copies per cell) [3], mini-exon gene sequences (100-200 copies per cell) [4], a repetitive sequence [5] and variable sequences of the ssu rRNA gene (20-40 copies per cell) [6]. Detection of the parasite DNA by qPCR has recently been implemented with the advantage of being able to detect between active

and subclinical infections and to quantitatively evaluate the parasitemia. However, qPCR has cost-related issues, requires a more complex infrastructure as well as qualified technicians and cannot be implemented in the field. Finally, the detection of the parasite through direct microscopic visualization or culturing of samples from macrophage-rich tissues (e.g. bone marrow) still represents the gold standard of VL diagnosis, even if is difficult to perform, invasive and not practical on a large scale [7].

As regards dogs, it is recommended, according to the symptoms and laboratory results, to combine the clinical diagnosis with specific techniques such as quantitative serology (IFAT) to analyse the homeral response (considering elevated antibody levels as diagnostic in dogs with compatible clinical signs and/or clinic-pathological abnormalities) [8]. In addition, along with evidence of the parasite in target organs (bone marrow, lymph nodes, skin, spleen), molecular biology methods are suggested to detect specific DNA through cytology and/or biopsy (conventional PCR, nested PCR, qPCR) [10]. Other diagnostic methods are of limited clinical use and require special equipment typical of research institutions: analysis of the cell immune response via a skin test or delayed hypersensitivity test and lymphocyte proliferation assay; culture in specific media and xenodiagnoses, both by isolating *L. infantum* in laboratory animals or in phlebotomine sand flies feeding on a suspect host [11]; and histopathology and immunohistochemistry on biopsies of target organs [10, 12].

In humans the parasitological diagnosis of VL is achieved following the microscopy visualization of amastigotes in smears stained with Giemsa, or following their culture in selective media, or the demonstration of their DNA through molecular methods in clinical samples (bone marrow, liver, spleen or lymph nodes). Specific anti-*Leishmania* antibodies are detectable in almost any immunocompetent individual with clinical VL, while they may be un- detectable or present at very low titres in patients with VL and simultaneous HIV/AIDS or other severe immunosuppressive conditions. IFAT or ELISA are the most common serological techniques. The immunochromatographic strip test using rK39 antigen is a rapid diagnostic test that can be used for the early diagnosis of VL. Serology is not useful to detect CL, as results are usually negative [7,13]. Quantitative measures of parasite DNA (qPCR) in peripheral blood can be helpful for measuring the initial parasite load and for monitoring responses to treatment. To confirm a diagnosis of CL, the parasite needs to be detected (microscopy or PCR) in aspirates or biopsies obtained of the skin lesion margins.

In conclusion the diagnosis of *Leishmaniosis* is complex and multiple strategies need to be used both for the human and animal diagnosis. In order to perform an accurate diagnosis is essential to know the performance of each diagnostic test and their more suitable niche of application. More universal diagnostic flowcharts should be promoted to detect these parasites under field and laboratory conditions in human and animal samples.

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