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Seroprevalence and molecular detection of *Leishmania infantum* in cats from Central Italy

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Background

Leishmaniasis is a neglected vector-borne disease causing an estimated 300,000 new cases and about 20,000 deaths in humans each year. In Italy, *Leishmania infantum* is the most important species of zoonotic concern, with dogs as the main domestic reservoirs and phlebotomine sand flies as vectors. Where canine leishmaniosis is endemic, cats are often exposed to the parasite. In Italy, an overall cumulative *L. infantum* prevalence of 3.9% was recorded in cats by serology and by qPCR, with a higher rate (10.5%) in southern regions as a result of the favorable geographical climate conditions that allow the presence and abundance of sand fly vectors [1]. In central Italy, feline leishmaniosis was scantly investigated with two reports from Tuscany with 0.9% and 2.5% of seroprevalence [2, 3]. The aim of this study was to assess the presence of *L. infantum* in a feline population from Central Italy (Latium region) using serological and molecular methods.

Materials and Methods

A total of 215 serum and blood samples were collected from cats admitted at the CDVet Research Laboratory (Rome, Italy) for routine controls. Detailed information about sex, age, and lifestyle was recorded for each animal. Sera were examined using three different serological tests for the detection of specific IgG against *Leishmania* using an anti-cat IgG conjugate: i) an indirect home-made immunofluorescence antibody test (IFAT); ii) a commercial IFAT (MegaFLUO Leish,Megacor Diagnostik GmbH); iii) a home-made enzyme immunoassay (ELISA) following the protocol by Alcover et al., 2021 [4]. The cut-off point (OD = 1.12 was established by calculating the mean value + 3 standard deviations (3SD) of 30 serum samples obtained from negative controls. The cut-off dilution of 1:80 was applied for both IFAT tests, following the LeishVet guidelines. Samples were classified as positive or negative for *Leishmania* when at least two of the tests yielded a positive or negative result.
Genomic DNA was extracted from blood samples and tested by qPCR (*Leishmania* all species Genesig® standard kit, UK). *Leishmania*-positive samples by qPCR were successively amplified by conventional PCR using primers L5.8S/LITSR targeting a partial region of the ITS1 (~300bp), and amplification run as described elsewhere [5]. Amplicons were purified and sequenced, in both directions, using the same primers as for the DNA amplification protocol. Sequences were compared with those available in GenBank using the BLASTn tool [6].

Results

Overall, antibodies against *L. infantum* were found in 11 of the 215 (5.1%) examined cats, with antibody titres ranging from 1:80 (45%), 1:160 (18%), 1:320 (18%), 1:640 (9%), 1:1280 (9%). No significant association was found with age, sex, lifestyle, or other co-infections. *Leishmania* DNA was found in the blood of one animal, which showed antibody titres of 1:640. The infected cat was under immunosuppressive therapy for previous intestinal lymphoma. The BLAST analysis of the amplicons revealed a high identity (98-100%) to *L. infantum* sequences deposited in GenBank.

Conclusions

Our preliminary study highlights the presence of *L. infantum* in 5% of the examined cats, in complete absence of historical features and physical signs compatible with the disease. The employment of different tests is an important critical issue influencing the different results in serodiagnosis. Thus, standardization of procedures for a prompt diagnosis of *L. infantum* in cats is crucial for a better understanding of the epidemiology and of the potential role of these animals as reservoirs of leishmaniosis.

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