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PhD THESIS

**Canine and feline leishmaniosis in Crete, Greece** (Abstract)

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# ABSTRACT

Leishmaniosis is a parasitic zoonotic disease of great importance, caused by protozoa of the genus Leishmania, transmitted primarily through the bites of female phlebotomine sandflies (Gramiccia et al. 2011). This PhD thesis provides a thorough overview of the disease in the landscape of Heraklion, Crete, focusing on its etiology, pathogenesis, transmission dynamics, clinical manifestations, diagnostic methodologies, therapeutic protocols, and preventive strategies in both humans and animals. In dogs, the primary domestic reservoir, the disease is predominantly caused by *Leishmania infantum*, and manifests in a polymorphic clinical spectrum ranging from asymptomatic infection to severe systemic illness (Solano-Gallego L. et al., 2009). The main vectors in Crete include *Phlebotomus neglectus* and *P. similis* (Ligda P. et al., 2023), while a newly identified species, *Ph. creticus*, may represent a novel vector of epidemiological concern (Dvorak et al., 2020). In case of humans, there are three forms of leishmaniosis including cutaneous, mucocutaneous, and visceral variants, with visceral leishmaniosis (VL) being the most severe (cdc.gov). Diagnostic approaches rely on cytology, serology (e.g., IFAT, ELISA), PCR, and histopathology, with PCR providing the highest sensitivity for detecting active infection (Guerin Philippe et al., 2002; Nieto C.G. et al., 1992; Solano-Gallego Laia et al., 2009; Tryphonas L. et al., 1977). Therapeutic options vary between species and disease severity, commonly involving allopurinol, miltefosine, meglumine antimoniate, and liposomal amphotericin B, though treatment challenges persist due to toxicity, drug resistance, and the lack of parasitological cures (Solano-Gallego Laia et al., 2011, Leishvet guidelines). Prevention in animals emphasizes vector control through insecticides and repellents (collars and spot-on substances), vaccination (Letifend®), and owner education. Furthermore, domperidone (Leishguard®) is a new substance used for the treatment and the prevention of the disease (LeishVet guidelines). The disease's endemicity in Crete, its emerging vectors, and the potential for vertical and venereal transmission in dogs, alongside increasing feline and human cases, underscore the importance of integrated, One Health-focused surveillance and control strategies.

The first part of the thesis consists of one chapter which offers a current overview of the scientific literature on the general aspects of canine, feline and human leishmaniosis, focusing on the etiologic agent, the pathogenesis, the vectors and the receptive hosts, the pathogenesis and immunity in animals with Leishmaniosis, the transmission of the disease, the clinical study in canines and felines, the diagnostic methods of canine and feline Leishmaniosis, the therapeutic protocols used in for the disease and the preventive methods used.

The second part (II original research) includes 5 chapters focused on assessing, the environmental particularities in the study of canine and feline leishmaniosis on the island of Crete correlated with the use of diagnostic tests, creating an overview of clinical and paraclinical tests for canine and feline leishmaniosis, for veterinary practitioners, the prevalence of *L.infantum* in the feline population of Heraklion, Crete, the canine Leishmaniosis in Crete, Greece: Epidemiology, Diagnosis, Prevention, and Therapeutic Protocols of the Disease, and the correlations between clinical manifestations, hematological and biochemical parameters, and IFAT titer in dogs infected with *Leishmania infantum* in Heraklion, Crete. This research was conducted on the island of Crete, Greece and the cited references conclude the thesis with 244 titles.

In the original research section, the working hypothesis and the research objectives are initially presented. Then, the island of Crete is described in relation to the use of diagnostic tests. The second chapter (II.2) presents the environmental particularities in the study of canine and feline leishmaniosis on the island of Crete correlated with the use of diagnostic tests. In this study, a standard protocol was used for each patient that entered the clinic with symptoms of leishmaniosis. Firstly, a clinical examination was done, and the questionnaire (annex 1) was completed by the owner/caregiver. Then, a rapid test was effectuated in order to confirm the disease. In most cases a quadruple test was used in order to evaluate if other vector borne diseases were present (erlihiosis, anaplasmosis, dirofilariosis). If the test was positive, we proceeded with blood test and analyzed the hematological and biochemical profile. For the majority of the patients, the Indirect Immunofluorescence Method (at a specialized laboratory in Athens) for determining the IgG antibody titer was done. Finally, Real-time PCR (qPCR) was used in order to evaluate the presence of the disease in blood or conjunctival SWABs. Moreover, in this chapter the working technic and the principle of method for each procedure is described and the normal values of the hematological and biochemical profile are given.

The following chapter, provides a comprehensive overview of the clinical and paraclinical diagnostic approaches for canine and feline leishmaniasis, tailored to veterinary practitioners. Diagnosis relies on an integrated approach including epidemiological data, clinical examination, hematological and biochemical profiles, immune response markers, and specialized diagnostic techniques. In-clinic tests such as immunochromatographic assays offer rapid results, while advanced methods—including cytology, histology, serology (ELISA, IFAT), and molecular diagnostics (PCR, qPCR)—enhance diagnostic precision and treatment monitoring. Recent advances highlight promising biomarkers and novel diagnostic tools like plasmonic ELISA, multiplex flow cytometry, and CatLeish-PCR. These innovations aim to improve sensitivity, specificity, and early detection of disease. The review emphasizes the importance of diagnostic strategies, considering animal history, regional prevalence, and clinical presentation, to ensure effective disease management and control.

The third chapter (II.3) is an original work and is about Feline leishmaniosis (FeL) in Crete. It is the first work about feline leishmaniosis in the island of Crete. FeL is primarily caused by *Leishmania infantum*, and it is an emerging zoonotic concern in Mediterranean regions. This study aimed to assess the prevalence of *L. infantum* among domestic and stray cats in Heraklion, Crete, a known endemic area. A total of 253 cats were sampled between 2018 and 2023 from urban and rural areas. Blood and conjunctival swab samples were analyzed using real-time quantitative PCR. The overall positivity rate was 5.9%, with higher prevalence among outdoor-living and male cats. Notably, infections were detected in both blood and swab samples, underscoring the utility of combining non-invasive diagnostic techniques for epidemiological surveillance. The study highlights the role of cats as potential secondary reservoirs and emphasizes the importance of regular monitoring, vector control, and public awareness, especially in high-tourism areas. Broader preventive strategies are needed to mitigate the spread of *L. infantum* and protect both animal and public health.

In the fourth chapter (II.4), the aim was to evaluate the epidemiological status, clinical manifestations, diagnostic methods, and treatment strategies for CanL in Heraklion, Crete. A total of 94 dogs examined between 2017 and 2022 were included, with clinical, hematological, biochemical, serological, and molecular data collected and analyzed. Most dogs showed systemic signs such as apathy (91.5%), weight loss (89.4%), and lymphadenopathy (79.8%), while 72.1% presented cutaneous lesions. Laboratory abnormalities included anemia, thrombocytopenia, hyperproteinemia, and elevated renal and hepatic biomarkers. Diagnosis was confirmed using serological rapid tests and indirect immunofluorescence (IFAT), while qPCR on blood and conjunctival swabs demonstrated higher positivity in swabs (90.7%) than blood samples (76.7%). The treatment of choice involved combinations of allopurinol with meglumine antimoniate or miltefosine, with domperidone as adjunctive therapy. This study confirms the endemic nature of CanL in Crete, underlining the necessity for regular monitoring, vector control, and public health awareness. Molecular diagnostics, particularly using conjunctival samples, proved to be highly effective for epidemiological screening.

In the fifth chapter (II.5), the aim was to reinforce the significant role of IFAT titers in the clinical management of canine leishmaniosis, demonstrating a strong positive correlation between antibody levels and disease severity. Elevated titers are indicative of a heightened immune response and greater parasitic load, often associated with more pronounced clinical and laboratory abnormalities. These findings underscore the diagnostic and prognostic value of serological testing, particularly IFAT, in staging the disease and guiding therapeutic decisions. Integrating IFAT results with clinical and laboratory parameters enables a more individualized and effective approach to treatment and monitoring. Further longitudinal research is warranted to elucidate the temporal relationship between antibody dynamics and disease progression, ultimately contributing to improved disease control and outcomes in both veterinary practice and public health contexts.

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