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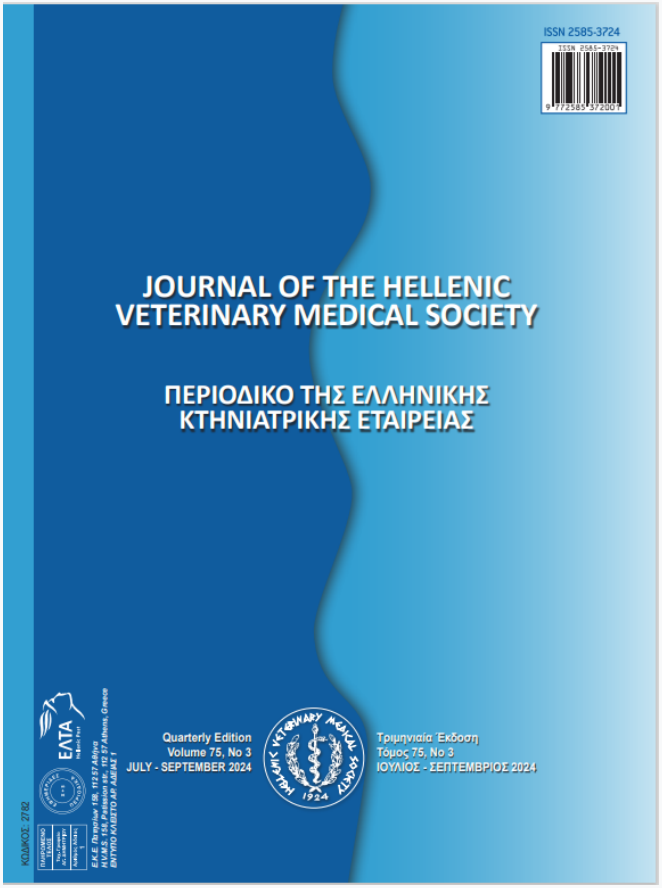


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Research article
Ερευνητικό άρθρο

# Prevalence of *Leishmania infantum* in felines from Heraklion region, Crete

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**ABSTRACT:** Greece is recognized as an endemic region for various species of *Leishmania*, a genus of parasitic protozoa transmitted through the bites of infected female sandflies. This indicates that the country serves as a natural habitat for this parasite, which is infecting both humans and animals. This study focuses on Heraklion, Crete, one of Greece's largest cities, where there is currently no available data on the prevalence of leishmaniosis in the feline population. While cases of canine leishmaniosis have been documented in various parts of the island, there have been no reported cases of *L. Infantum* infection in the felines in Heraklion. This study primarily investigates the prevalence of leishmaniosis in cats as a means of detection in this species in Heraklion. To accomplish this, between 2018 - 2023, EDTA-blood samples and conjunctival swab samples were collected from a total of 253 feral cats. The cats were divided into two groups, depending on the location they lived (i.e., areas out of the city center or close to/in the city center): the initial group consisted of 112 cats, while the latter group comprised 141 cats. Both groups encompassed cats of various ages, habitats, and both genders. The samples were stored at -18 °C and analyzed with TaqMan real-time quantitative PCR for the presence of *Leishmania infantum* DNA. Out of the cat population included in the study, 15 were tested positive for *L. Infantum* infection (5.93%). Despite the lower prevalence of leishmaniosis compared to dogs, it should be taken into account, especially for differential diagnosis. In conclusion, to the best of our knowledge, this is the first study that confirms the presence of FeL in felines in Heraklion.

***Keywords***: Feline leishmaniosis; *Leishmania* spp.; Greece; Heraklion; prevalence

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## INTRODUCTION

Feline leishmaniosis (FeL) is a disease with public health significance. While cats might not exhibit severe symptoms compared to dogs, they could carry the parasite and serve as a potential source of infection. Studying and managing feline leishmaniosis is important in order to eliminate the spread of the disease and prevent potential human and other mammal infections.

In the Mediterranean basin, various species of female sandflies are vectors of *Leishmania* spp., thus infecting the definitive hosts (vertebrates), through the bite during feeding time. As definitive hosts, other than humans, in the Mediterranean area, felines, rodents and equines have been also reported (Kouam et al., 2010; Garcia et al., 2014; Tsokana et al., 2016; Limeira et al., 2019; Gazzonis et al., 2020; Martin - Sanches et al., 2021; Tzanis et al, 2021). As a result, the distribution, occurrence in animal populations, and the spread of *Leishmania* species are closely associated with the presence and behavior of these particular vectors (sandflies). The transmission of this parasitic protozoa to cats is not completely elucidated. Sandflies have been indirectly observed to feed on cats and acquire the parasite, through experimental documentation. (Maroli et al., 2007, da Silva et al., 2010). However, based on existing information about the transmission in other vertebrates, there is no doubt that it occurs through the bite of female sandflies.

Feline leishmaniosis is caused by at least 5 species of *Leishmania*: *L. mexicana*, *L. venezuelensis, L. braziliensis*, *L. amazonensis*, and *L. infantum* (Pennisi et al., 2015). Most commonly, the species responsible for the feline infection is *L. infantum*. Similar to dogs, it is a vector-borne disease of significant importance for both public and animal health (Pereira and Maia, 2021). In the past, it was supported that sandflies had a preference regarding their food source. However, recent studies have shown that also cats are a food source for sandflies. Specifically, infected cats can successfully transmit *L. infantum* to *Phlebotomus perniciosus* sandflies, where the parasite continues its life cycle (Maroli et al., 2007; Gonzalez et al, 2017). In addition, various studies have demonstrated that *L. infantum* circulates among feline populations in the Mediterranean Basin, such as Greece, Portugal, Turkey, Cyprus, Spain, Italy and Albania (Mancianti et al., 2004; Diakou et al., 2009; Maia et al., 2010; Silaghi et al., 2014; Can et al., 2016; Attipa et al., 2017; Montoya et al., 2018; Iatta et al., 2019; Morelli et al., 2019). Current climate changes boost the breeding and spread of sandflies, leading to more frequent transmission of leishmania infection to vertebrates. Greece is among the top tourist destinations, and travelling with pets is very common, the correlation between animal travel and the spread of the disease from endemic to non-endemic areas is of great importance (Spada et al., 2020; Diakou et al., 2017; Maia et al., 2015). In these cases, continuous epizootiological monitoring plays a crucial role in the protection of both human and animal welfare.

Continued research regarding leishmaniosis is essential in order to improve diagnostic methods, treatment protocols, and preventive measures, specifically tailored to the feline population. Under this perspective, the aim of this study was to enhance the understanding of the prevalence of *L. infantum* and its transmission dynamics among the feline population of both rural and urban environments in Crete Island.

## MATERIALS AND METHODS

### Study design

The study was conducted on the island of Crete, the fifth biggest island of the Mediterranean Sea, and more specifically in Heraklion, in the north part of the island. As an island, Crete is mostly mountainous with a few lakes and rivers. It is characterized by a Mediterranean and semi-arid climate, and it has a mainly humid atmosphere and mild winters.

A total of 253 cats from various areas of Heraklion were included in the study. Those 253 cats were divided into two groups. The first group consisted of 112 cats originating from areas outside the city center, including the villages: Voutes, Gournes, Giofiro, Finikia, Staurakia, Dafnes, Kallithea, and Kartero, and the second group consisted of 141 cats originating from areas in close proximity to the city center or from the center. Information regarding the gender, lifestyle, and age of each animal was recorded.

In total, 106 male and 147 female cats were included in the study. One hundred fifty-six cats of both genders lived outdoors, 57 cats lived indoors and 34 cats lived both indoors and outdoors. The age of the cats ranged from six months to over eight years old. As for the outdoor-living and stray cats the age was approximately estimated based on their denture. The majority of the cats included in the study were brought to the clinic for castration. As for the aggressive ones', special traps were used by their owners or

caretakers in order to capture them.

Owners or caretakers of the animals provided consent for sample collection and analyses.

**Sample collection**

Blood samples were collected in EDTA tubes from all the cats, from the jugular or cephalic vein, along with swab samples from their ocular conjunctiva. The samples were stored at -18 °C, until analyses. Genomic DNA was extracted from the EDTA-blood samples and the conjunctival swabs using the DNeasy Blood and Tissue kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's instructions.

Blood and conjunctival DNA samples were tested for the presence of *L. infantum* DNA using a TaqMan real-time quantitative PCR (qPCR) assay that targeted a 120-bp fragment of the kinetoplast minicircle DNA, as previously reported (Francino et al., 2006). A positive (reference DNA sample from blood and conjunctival swab, respectively) and a negative (PCR grade water) control were included in each qPCR run.

**Statistical analysis**

For carrying out the statistical analyses, the IBM SPSS Statistics data editor program was used. The following parameters were evaluated for each cat included in the study: age, gender, habitat, and any co-existing conditions. The cats were divided into two groups. The first group consisted of 112 cats originating from areas outside the city center, while the latter included 141 cats from areas in close proximity to the city center or from the city center itself.

## RESULTS

The 253 cats included in the study were further divided into two groups as mentioned above. Out of the 112 cats that lived out of the city center, 51 were males and 61 were females (see Table 1). Seventy-seven (77) lived outdoors, 25 indoors and 10 lived both indoors and outdoors (Table 2). The age of the cats included in the study is presented in Figure 1, and it ranged from six months to eight years old.

A total of 11 cats in the first study group were positive for the presence of *L. infantum* either in blood or in conjunctival swab samples or in both samples (blood and conjunctival SWAB). Precisely, three cats had a positive conjunctival swab sample, five cats had a positive blood sample, and three of them were found positive both in blood and swab sample analyses (see Figures 3 and 4). Out of the 11 positive cats, seven were males and four females, only one cat lived indoors, nine lived outdoors, and one lived both indoors and outdoors. It is worth noting that none of the infected with *L. infantum* cats had any other systemic disease.

The second group of the study consisted of 141 cats living in close proximity to the city center/to the city center, 86 were females and 55 were males (see Table 6). Thirty-two lived indoors, 24 lived both indoors and outdoors, 79 were outdoors, and 6 cats were found injured on the street due to a car/motorcycle accident (see Table 7). The age of the cats in this group ranged from six months to over eight years old (see Figure 4).

Out of the 141 cats, the conjunctival swab yielded positive results for three of them (see Figure 5), one cat tested positive both in blood and swab sample, while no positive results were obtained when analyzing the blood samples (see Figure 6). These three positive swab samples were from two male cats who lived outdoors, and from a female who lived both indoors and outdoors. The cat with the positive blood and swab sample was a female that lived outdoors.

**Tabel 1 -** Gender of cats in group 1

| | | Frequency | Percent | Valid Percent | Cumulative Percent |
|---|---|---|---|---|---|
| Gender | female | 61 | 54.5 | 54.5 | 54.5 |
| | Male | 51 | 45.5 | 45.5 | 100.0 |
| | Total | 112 | 100.0 | 100.0 | |

**Tabel 2 -** Habitat of cats of group 1

| | | Frequency | Percent | Valid Percent | Cumulative Percent |
|---|---|---|---|---|---|
| Habitat | Indoor | 25 | 22.3 | 22.3 | 22.3 |
| | indoor/outdoor | 10 | 8.9 | 8.9 | 31.3 |
| | Outdoor | 77 | 68.8 | 68.8 | 100.0 |
| | Total | 112 | 100.0 | 100.0 | |

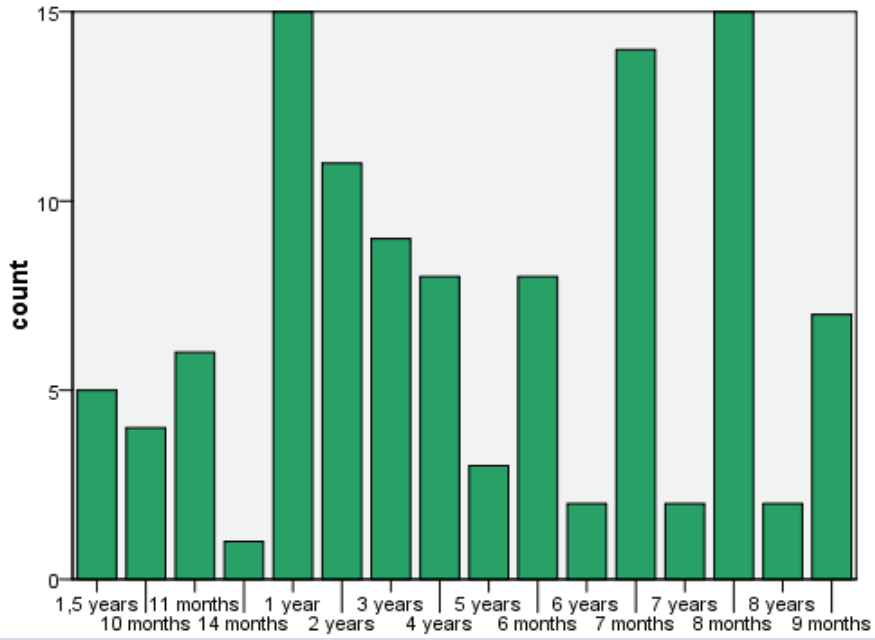


**Figure 1.** Age of the cats in study group 1 (cats lived in areas out of the city center).

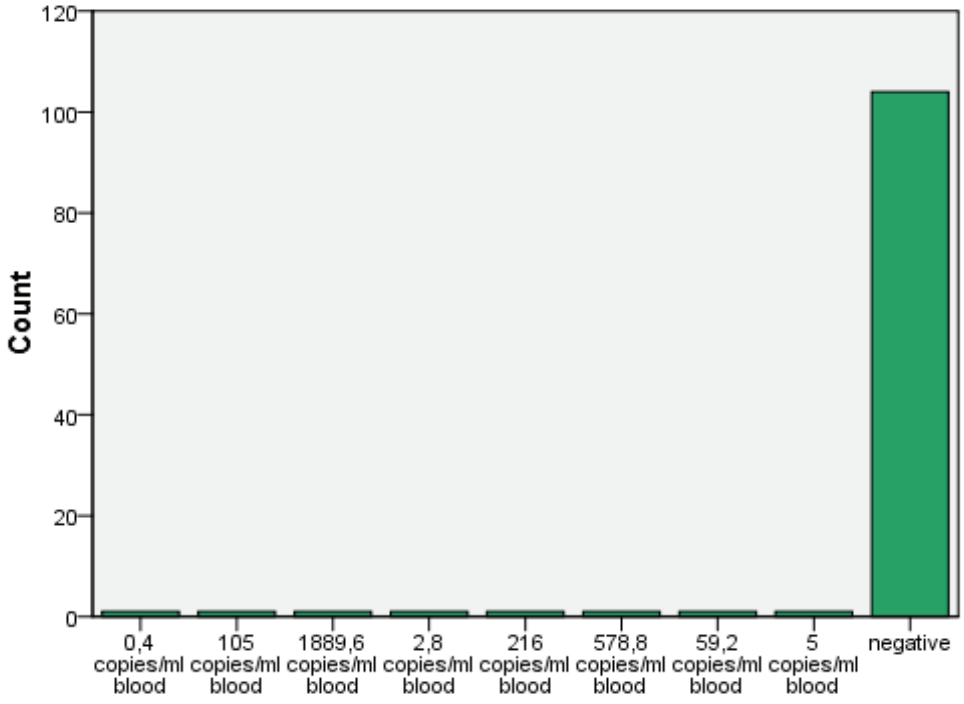


**Figure 2.** Results of real-time qPCR analyses of blood samples of study group 1 (cats lived in areas out of the city center). The x axis, refers to the parasitic load of the positive samples.

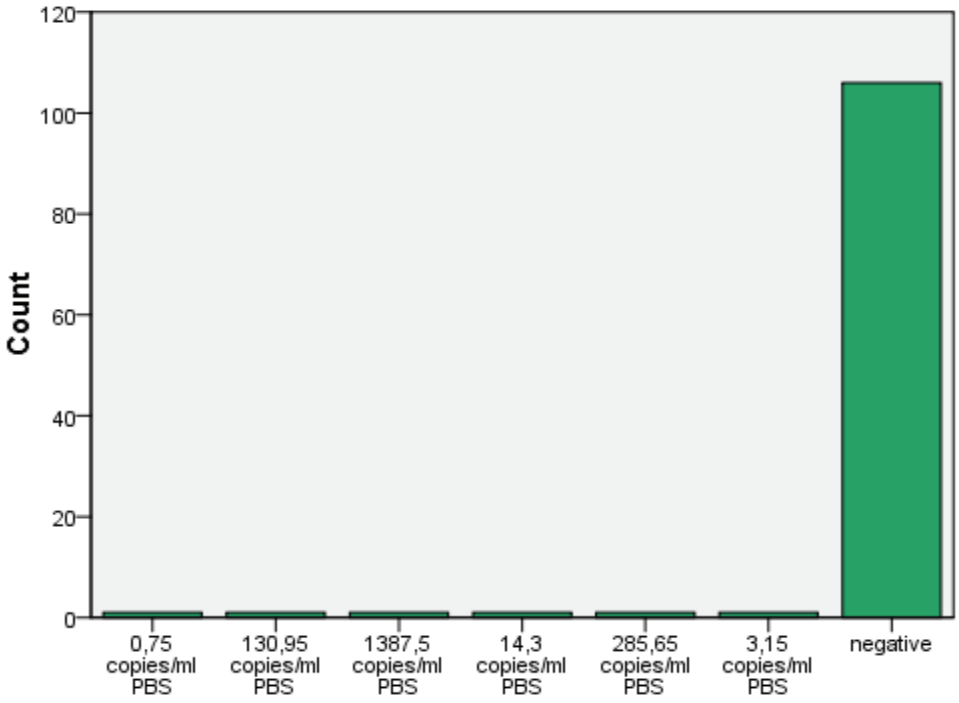


**Figure 3.** Results of real-time PCR analyses of conjunctival swab samples of study group 1 (cats lived in areas out of the city center). The x-axis refers to the parasitic load of the positive samples.

**Tabel 6 -** Gender of patients of group 2

| | | Frequency | Percent | Valid Percent | Cumulative Percent |
|---|---|---|---|---|---|
| Gender | Female | 86 | 61.0 | 61.0 | 61.0 |
| | Male | 55 | 39.0 | 39.0 | 100.0 |
| | Total | 141 | 100.0 | 100.0 | |

**Tabel 7 -** habitat of cats of group 2

| | | Frequency | Percent | Valid Percent | Cumulative Percent |
|---|---|---|---|---|---|
| Habitat | Indoor | 32 | 22.7 | 22.7 | 22.7 |
| | indoor/outdoor | 24 | 17.0 | 17.0 | 39.7 |
| | Outdoor | 79 | 56.0 | 56.0 | 95.7 |
| | Stray | 6 | 4.3 | 4.3 | 100.0 |
| | Total | 141 | 100.0 | 100.0 | |

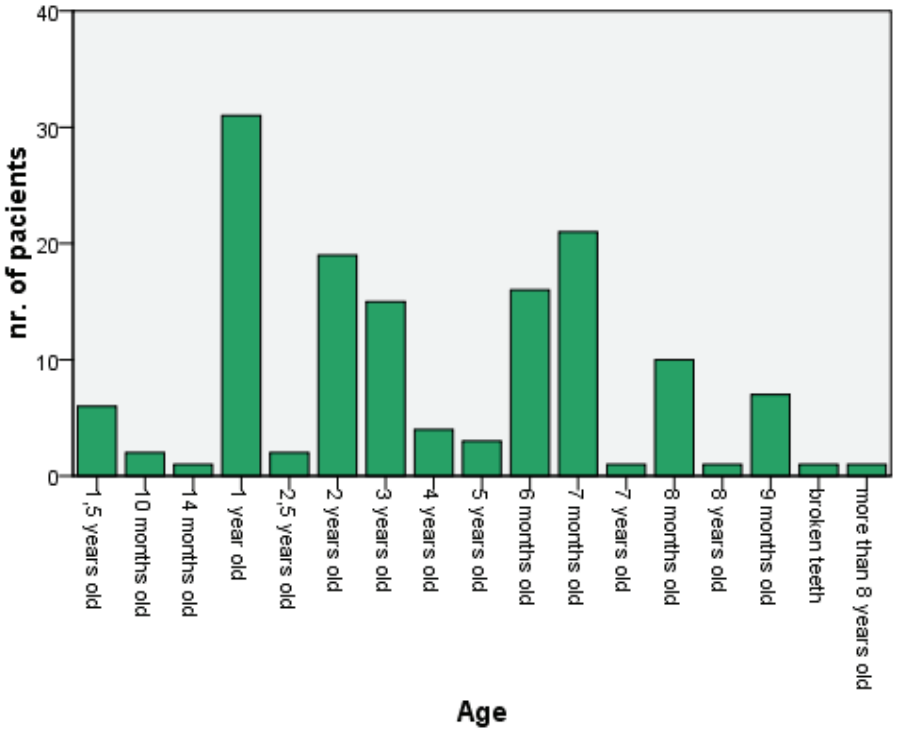


**Figure 4.** Age of the cats in study group 2 (cats lived in areas in close proximity to the city center or to the city center).

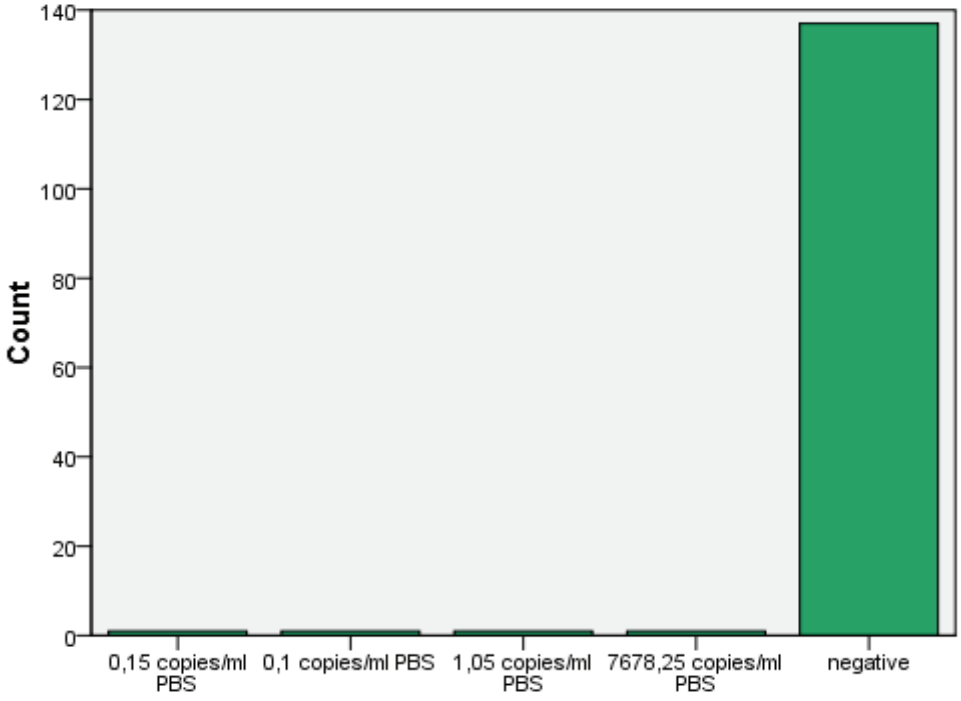


**Figure 5.** Results of real-time qPCR analyses of conjunctival swab samples of studygroup 2 (cats lived in areas close to the city center or to the city center). The x-axis refers to the parasitic load of the positive samples.

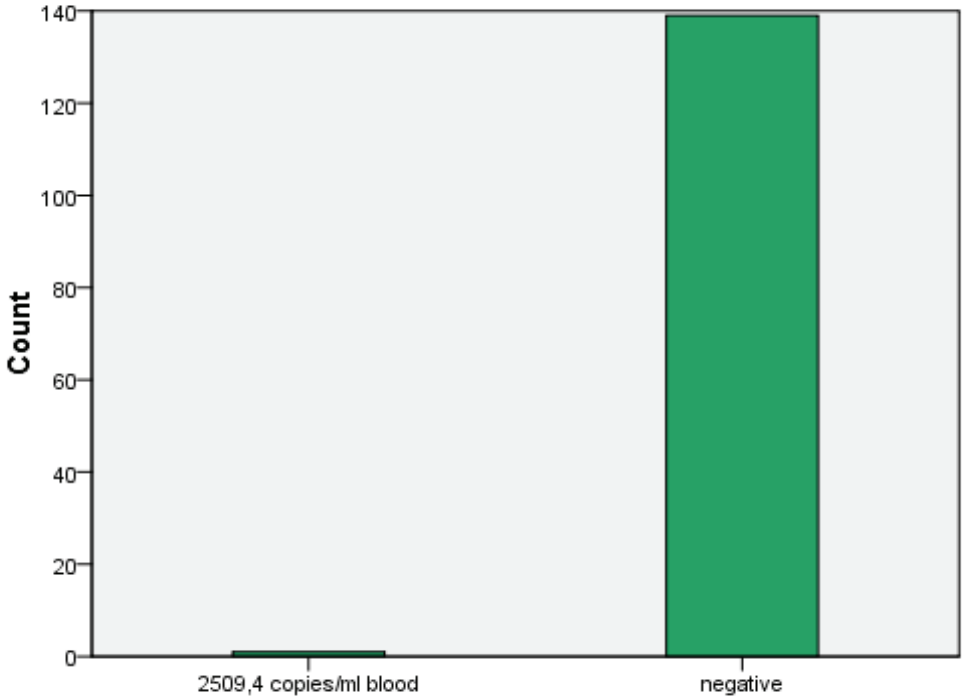


**Figure 6. -** Results of real-time qPCR analyses of blood samples of study group 2 (cats lived in areas close to the city center or to the city center). The x-axis refers to the parasitic load of the positive samples.

Overall, out of the 253 cats included in the study, a positivity of 5.93% for *L. infantum* infection was recorded.

## DISCUSSIONS

This study was carried out in order to examine the presence of *L. Infantum* in the feline population of Heraklion, Crete. It has been demonstrated that felines can be involved in the transmission of leishmaniosis, most likely as secondary reservoirs (Maroli et al., 2007; Maia et al., 2011 Pennisi et al.; 2015 Morelli et al., 2020). According to experimental infections, it has been shown that cats are less sensitive to the parasite than dogs (Maroli et al., 2007; Maia et al., 2011; Pereira and Maia,2021). Therefore, the disease manifests more discreetly in felines, although some infected cats may develop clinical signs and/or laboratory abnormalities like those encountered in canine leishmaniosis (Pennisi et al., 2013; Chatzis et al., 2014). *L. infantum* infection in felines is rare, although it has already been described in Mediterranean Europe, including Greece, Italy, Spain and Portugal (Diakou et al., 2009; Sherry et al., 2010; Vihlena et al., 2013; Chatzis et al., 2014; Morelli et al., 2020; Nascimento et al., 2022).

To the best of our knowledge, limited data are available regarding the epidemiological situation of *L. infantum* infection in cats on the island of Crete. The data obtained from the present study adds new information about the epidemiological situation of the disease in the feline population of Heraklion. Cats found positive for *L. infantum* were born on the island and have never travelled abroad, indicating the presence of the parasite on the island, which is not surprising, as it is known that Crete is endemic for *Leishmania*, with documented cases in both humans and animals (Morelli et al., 2020). A previous study conducted in Greece (Mykonos Island), including 153 cats, revealed a seroprevalence of 2.97% (Morelli et al., 2020). Overall, and as reported by Symeonidou et al. in 2023, the presence of the parasite has been also demonstrated in Chania (5 out of 34 cats found positive for *L. infantum* infection - 14.7%) (Symeonidou 2023).

The presence of parasitic DNA in both blood and swab samples demonstrates that cats are frequently exposed to vectors. Real-time qPCR used in this study, on conjunctival swabs and blood samples, takes advantage of the high sensitivity of the method (Qiagen GmbH, Hilden, Germany) in combination with its non-invasive nature for the screening of leishmania infection in cats, detecting the infection at an earlier stage compared to other techniques (i.e., those detecting antibodies).

Comparing the positivity of FeL in both study groups and in both blood and swab samples, it was demonstrated that almost half of the total positive results referred to blood sample analyses and the other half to swab samples. Most of the positive cats lived outdoors (80.0%), 13.3% lived both indoors and outdoors, and 6.7% lived indoors. The age of positive cats ranged between 6 months old and 4 years old in both groups. Regarding gender, 60.0% of the positive cats were males and 40.0% were females, in comparison to Morelli's study (2020), where most of the positive cats were females.

The results obtained in this study demonstrate that outdoor or/and indoor-living and stray cats can be exposed to *L. infantum* infection. This is probably a result of the limited use or even lack of use of antiparasitic treatments, for instance in cats living in uncontrolled groups. One possible explanation for this could be that most outdoor-living cats are not easily accessible, as they can be aggressive, making it difficult for their caregivers to apply parasiticides of any form. Another reason could be the fact that most caregivers have many animals, and due to economic limitations, they may not be able to provide the cats with the appropriate protection. It should also be taken into consideration that the number of stray cats that do not receive any medical care is much higher compared to the ones that receive at least the minimum of it.

In endemic areas, it is expected that felines will become infected and serve as a persistent source of infection for vectors. The ability of sandflies to feed on cats and acquire *L. infantum* has been indirectly documented in experimental studies (Maroli et al., 2007; da Silva et al., 2010; Gonzales et al., 2017).

Furthermore, residing outdoors in endemic areas, in the same habitat as dogs infected with leishmaniosis, also increases the percentage of infection in cats. Dogs infected with *L. infantum* are the major source of infection for sandflies, which can also bite humans and other mammals, such as felines. Cats, especially those in free-roaming colonies, may become highly exposed to vector-borne diseases such as leishmaniosis due to their independent lifestyle. The most recent study conducted by Lidga et al. (2023) in Crete about sandfly fauna, demonstrated that various sandfly species are circulating on the island, with the most predominant being: *Phlebotomus neglectus* and *Sergentomyia minuta*, followed by *P. tobbi*; *P. perfiliewi, P. similis, P. simici, P. mascittii, P. papatasi and S. dentata*. However, according to the molecular analyses conducted, no *L. infantum* DNA was detected in any of the sandflies collected. Also, Dvorak et al. (2020), found the above-mentioned sandfly species in Crete and precisely: *P. neglectus, P. simici, P. papatasi, P. similis, P. minuta,* while *P. tobbi, P. alexandri, P. killicki and Sergentomyia minuta* were found in a smaller sample size.

To summarize, it is essential to regularly monitor the animals living in tourist areas, such as Crete, as the movement of infected animals poses a substantial risk of spreading pathogens. In fact, not only pets travelling to enzootic areas can become infected with new pathogens, but they can also introduce pathogens from enzootic to non-enzootic areas when they return back (Diakou et al., 2017; Kostopoulou et al., 2020). Therefore, control measures are fundamental in preventing and managing enzootic infections in both dogs and cats. This involves scheduled treatments and preventive measures using broad-spectrum and effective antiparasitic formulations. At the same time, it is essential to educate the public and public health authorities about the potential risks associated with free-roaming cats to encourage the safe movement of both people and pets (Diakou et al., 2017).

## CONCLUSIONS

The present study carried out in Heraklion, Crete, regarding the prevalence of *L. infantum* infection in 253 cats of different ages, genders and living conditions, in 2018-2023, has revealed that molecular diagnosis using blood and conjunctival swab is recommended as a screening method for epidemiological analysis. Infected felines are a potential risk for both humans and other mammals, as they are considered a reservoir of the disease. The results obtained (positivity of 5.93%) are probably a result of the limited use or even the lack of use of antiparasitic treatment in the cats of the study area. More drastic measures should be taken, and further epidemiological studies should be performed in the feline population in Greece, in order to obtain a better understanding of the situation of *L. infantum* infection.

## CONFLICT OF INTEREST

None declared

## ACKNOWLEDGEMENTS

None

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