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First Serological detection of *Leishmania* spp of domestic and stray cats in Al-Qadisiyah Province, Iraq

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Abstract Leishmaniasis is a major mosquito-borne disease due to protozoa of Leishmania genus which spread by sand flies' bites. This study was conducted to detect Leishmaniasis in domestic and stray cats by using serological test (Indirect Enzyme-Linked Immunosorbent Assay -ELISA) and to determine the effects of sex, age and months on the infection rate in Al-Qadisiyah Province. A total of 142 samples were collected during the period from the first of July to the end of December/ 2024. Leishmaniasis IgG antibodies were detected rate 20.42% (29/142) of the cats by indirect ELISA. High infection rate was recorded in stray cats 28.37% (21/73), compared to domestic cats 11.76% (8/68), with significant difference at P<0.05. the prevalence rate was in males 23.28% (17/73) and in females 17.39% (12/69) without significant difference at P≥0.05. Moreover, the highest infection rate in domestic cats was observed in the adult was 15.55% (7/45) while the lowest in kittens 4.34% (1/23), without significant difference at P<0.05. The prevalence was altered during various months studied where the highest and lowest rate was recorded (34.61%) and (4.54%) in September and July respectively, without significant difference at P≥0.05. For the first time, Leishmaniasis was recorded to infected stray and domestic cats in Iraq

Keywords: Al-Qadisiyah, Blood, cats, ELISA, Leishmaniasis

Introduction *Leishmania* a zoonotic human disease, is hyperendemic in subtropical and tropical areas of the world. Visceral leishmaniasis (VL) influences approximate 12 million individuals worldwide, with an incidence rate ranging from 0.2-0.4% (1). Leishmania spp. are spread by a variety of female sandfly species in the Mediterranean basin, which bite the definitive hosts (vertebrates) during feeding time (2). Although cats were once thought to be less vulnerable to L. infantum, they are now understood to be both competent hosts for the parasite and a source of blood for its sand fly vectors (3,4). Moreover, feline leishmaniasis (FeL) attributed to L. infantum has been recorded worldwide, precisely in the Mediterranean areas (5,6,7).

Hepatomegaly, lymphadenopathy, ulcers, and eye lesions and nodules are the most common clinical signs in these cases (8). Granulomatous inflammation with many amastigotes has also been seen in mucosal nodules, kidney, liver, spleen, and the eyes (9,10). cutaneous, mucosal, and visceral signs are among the disease's clinical manifestations. Systemic and high mortality participation of the organism was linked to visceral signs. Significant morbidity is often caused by the spread of parasites through other tissues, which is often linked to cutaneous or mucocutaneous symptoms (11). A comparative analysis of the complement system's activation in various hosts following stimulation with L. infantum revealed that cats were less susceptible to infection because their classical and alternative pathways were less activated, and their lectin pathway was more activated because of less C4b deposition (12).

Leishmania spp inveestigate in cats involved, L. braziliensis, L. amazonensis, L. venezuelensis, L. mexicana and L. infantum (13). various worldwide studies confirmed a high seroprevalence of FeL leishmaniasis was detected by IFAT and ELISA (14,15,16).

The reaction of antibodies presents in the sera with purified and soluble Leishmania antigens derived from in vitro culture is what drives ELISA (17). By ELISA, the approach has been extremely useful for the investigation of anti-Leishmania antibodies.

Materials and methods

Ethical approve

This study was approved for the animal care at the College of Veterinary Medicine, University of Al-Qadisiyah, Under the No. 4955 dated 17/11/2024

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Collection of samples

Using single-use syringes and needles, one hundred and forty-two blood samples were collected from the cephalic vein or jugular vein of stray and domestic cats randomly selected from the areas of Al-Qadisiyah Governorate from July to December 2024. And transferred to anticoagulant-free tubes. Then, the blood was centrifuged for 12 minutes at a speed of about 3000 rpm, and one ml of the serum was transferred to fine plastic tubes and saved at -20°C for serological examination. (19,20).

Indirect ELISA

In this study, an indirect ELISA test was used to detect IgG antibodies in the serum of the studied cats against leishmaniasis. The manufacturer's instructions for the ELISA test kit for the leishmaniasis were followed, and serum samples from all the studied cats were prepared with the kit materials and reagents. The optical density (O.D.) was measured using a microtiter plate reader (BioBase, China) at 450 nm within 20 min after analysis. The results were calculated using the generated standard curve by plotting the average O.D. originated for each of the standard concentrations on the horizontal (X) axis as well as the vertical (Y) axis concentration and the positive sensitivity result is 0.1 ng/ml (,21,22).

Results

The total infection rate of feline leishmaniasis in blood samples examined were 20.42% (29/142). Table (1) shows a higher infection rate of Feline Leishmaniasis in stray cats 28.37% (21/74), more than domestic cats 11.76% (8/70). with significant variance.

Table 1: Total infection rate of Leishmaniasis according to type of cats.

Cats	No. of examined samples	Positive	Percentage (%)
Stray	74	21	28.37
Domestic	68	8	11.76
Total	142	29	20.42
Chi|-Square(χ2)		6.01	
P value		0.014 (HS)	

HS: Highly significant difference at $p<0.05$

Infection rate according to sex

A higher infection rate was detected in males Cat 23.28% (17/75), while the lowest in females Cat 17.39% (12/71), without significant difference P > 0.05 (Table 2).

Table 2: Infection rate of Feline Leishmaniasis according to sex by using indirect ELISA.

Sex	No. of examined samples	Positive	Percentage (%)
Males	73	17	23.28
Females	69	12	17.39
Chi-Square (χ2)		0.759	
P value		0.384 (No)	

NS: No significant difference at $p<0.05$

Infection rate in domestic Cats according to age

Adult cats, which showed a high infection prevalence than kitten cats were recorded a lower rate. There was significant variability (Table 3).

Table 3: Infection rate of Feline Leishmaniasis according to age by indirect ELISA

Age	No. of examined samples	Positive	Percentage (%)
Kittens	23	1	4.34
Adults	45	7	15.55
Total	68	8	11.76
Chi-Square (χ2)		1.84	
P value		0.175 (NS)	

NS: No significant difference at $p<0.05$

Infection rate according to months

The infection rate of Feline Leishmaniasis was higher in September (34.61%), followed by October (29.62%), and there was low infection rate in December (9.52%), followed by July (4.54%) (Table 4).

Table 4: Infection rate of Feline Leishmaniasis according to months by indirect ELISA.

Months	No. of examined samples	Positive	Percentage (%)
July	22	1	4.54
August	22	5	22.72
September	26	9	34.61
October	27	8	29.62
November	24	4	16.66
December	21	2	9.52
Chi-Square (χ2)		9.85	
P value		0.079 (NS)	

NS: No significant difference at $p<0.05$

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Discussion

Leishmaniasis is a complicated fly-borne disease originated by different species of Leishmania that is distributed in 80 countries of South and Central America, , Asia and Africa , greater than 350 million individuals are calculated at risk of Leishmania (23) .The objective of this study was investigation the existence of against Leishmania antibodies based on serological techniques in stray and domestic cats in Al-Qadisiyah Province Iraq. This study is the first detection of leishmaniasis in cats from Iraq where the incidence of leishmaniasis was recorded at 20.42%. And agreement with a study in Greece 23.2% (24), in Iran 24.27% (25), in Portugal 24.7% (26), in southern Italy 25.8% (27), in center Italy16.3% (28), in Turkey 15.2% (29). and differed with the results of the study France 13.3% (30), in Italy 12.6% (31), in Jerusal 6.7% (32), in Pernambuco 3.9% (33), In Greece (Thessaloniki) 3.87% (34), 0% in Angola (35).

20.42% of examined cats were positive for antibodies against Leishmania using ELISA. The distribution of feline leishmaniasis was different between areas, and when comparing this investigation with other studies, the variables originated from various serological examinations and cross-reactions with other Leishmania species or may be due to different levels of distribution, types of feline populations involved in these studies, and validation of the serological techniques. (36).

The current study showed a higher infection rate of Feline Leishmaniasis in stray cats, 28.37% more than domestic cats, 11.76%. with found significant variance. And agreement with a study in the Mediterranean Basin is 11.9%, and domestic is 8%. recorded a high positive in sheltered cats in comparison with private-owned cats in the province of Paran´a, BziParaná, inreater positivity that founded in sheltered cats may be due to their continuous exposure to infected mosquitoes, as the shelter examined in previous in a previous study surrounded by open sewage and lack of basic sanitation as well as vegetation that supports the existence of vectors. Moreover, living together with infected dogs may pose the likelihood of feline leishmaniais (37).

The current study showed the highest infection rate was recorded in males at 23.28%, while the lowest in females was 17.39% without significant difference. This study found agreement with the study in cats from southern Iran. Female 12.33% and male 38.61% (38). The results of our study differ from the study of Italy and Greece male 2.1% and female 3.9%. (39). For behavioral and environmental reasons, they are more prone to feline leishmaniasis. Male cats are more likely to roam around and fight with other cats, which raises the possibility that they will get bitten by disease-transmitting insects. According to some research, sex hormones might influence how the immune system reacts, making males less immune to infection than females. in adult cats showed a higher infection prevalence of 15.15% compared to kittens, which have a lower positivity rate of 4.34%. There was a large variation in the age of the cats, and nearly in agreement with a study conducted in the Mediterranean basin, the result was 13% in adult cats and 9.3% in kittens (40).

unexpectedly, adult cats have the higher risk of infection with *L. infantum* in comparison with other groups of age (41). this study the infection rate of Feline Leishmaniasis was higher September (34.61%), and lower rate in July (4.54%). was different from, the study in northern Italy where it was recorded higher in August 33.3 %, and there was low infection rate in October 5.6%, (42).

Temperature, precipitation, and humidity all have a significant impact on the behavior and distribution of sand flies (43). By altering the number of sand flies or the air temperature, which indicates the parasite's development and growth in the vector host, climate change and ambient temperature can have an impact on the spread of Leishmania. As for our current study, it is an increase in the prevalence in cats that were sampled in September and October, due to the increased activity of the Phlebotomus species in Al-Qadisiyah Province of Iraq during these two months.

Conclusions

This study provides, for the first-time serological detection of Leishmania spp, in stray and domestic cats in Al-Qadisiyah Province, Iraq. Indicates that cats are regularly bitten by infected sand flies in and may have a potential reservoir role in the maintenance of L. spp. moreover, additional studies are required to determine the role of cats in the distribution of this parasite and regardless of if this species should be actually considered closely reservoir of current parasite.

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Conflict of interest

The authors declare that there is no conflict of interest of this paper.

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