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Paper

Canine Leishmaniasis in Eastern Algeria: Seroprevalence and Risk Factors

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Abstract

A seroepidemiological survey was conducted between 2021 and 2025 to assess *Leishmania infantum* infection in domestic dogs from urban and rural areas in three provinces of eastern Algeria: Batna, Oum El Bouaghi, and Biskra. Blood samples were collected from 347 dogs and tested for anti-*Leishmania* antibodies using an enzyme-linked immunosorbent assay (ELISA). The overall seroprevalence was 10.37%. Among seropositive dogs, 33.3% were asymptomatic, while 66.6% exhibited clinical signs, with emaciation being the most frequently observed symptom. Statistical analysis showed significant associations between seropositivity and several risk factors, including age, geographical area, clinical status, nutrition, province of origin, and lifestyle.

Keywords

Leishmania infantum, canine leishmaniasis, seroprevalence, riskfactor, Algeria, ELISA

Introduction

Canine leishmaniasis (CanL) is a vector-borne zoonosis caused mainly by the protozoan *Leishmania infantum*, widely recognised as one of the most concerning parasitic infections according to the World Health Organization (WHO). Endemic in the Mediterranean basin, West Asia, and South America, this disease represents a major public health issue due to the central role played by dogs as reservoirs of the parasite, facilitating transmission to humans via sand flies. In the Mediterranean region, canine prevalence has been estimated at 16.6%, with nearly 2.5 million dogs infected (Priolo et al., 2024).

A major challenge in the fight against this zoonosis is that the majority of infected dogs remain asymptomatic, while being potentially infectious to vectors. When presenting clinically, the disease can cause severe damage, particularly to the kidneys and blood, and even death in the absence of appropriate treatment. The management of CanL is therefore based on early detection of cases, identification of risk factors, and a detailed understanding of its epidemiological dynamics.

In Algeria, CanL is well established, particularly in the northern and northeastern regions. Particularly high prevalence

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rates have been reported in the wilayas of Sétif (69.17%), Bouira (62.5%) (Medkour et al., 2020), and Tiaret (68%) (Bia et al., 2022). Conversely, lower rates have been reported in Saïda (6%) (Tabet Aoual & Ammam, 2022) and in the Kabylie region (35.7%) (Medkour et al., 2019). A meta-analysis conducted over the period 2004–2022 estimated the average prevalence at 21.2% in the country (Touhami Khelifi et al., 2022). This spatial variability can be attributed to differences in ecological conditions, surveillance practices, and the diagnostic methods used.

Another meta-analysis conducted in the Maghreb region (Algeria, Tunisia, Morocco) on data collected between 1973 and 2022 revealed an overall prevalence of 21.14%, with 2,481 positive cases identified out of 11,736 dogs tested. These results show an upward trend in prevalence over time. (Baaziz et al., 2024)

Among the factors contributing to this re-emergence in Algeria, climate change and environmental transformations play a key role, favoring the geographical spread of vectors and the spread of the disease outside its traditional foci (Benikhlef et al., 2021; Boudrissa et al., 2012; Harrat, 2009).

Northeastern Algeria is historically recognised as an endemic focus for canine leishmaniasis (Adel et al., 2015), but several areas remain under-documented. In this context, this study aimed to estimate the seroprevalence of CanL and identify associated risk factors in dogs in several localities in eastern Algeria. The method used is based on an ELISA (enzyme-linked immunosorbent assay) test, commonly used for the detection of specific antibodies. This approach provides useful data for a better understanding of the epidemiology of canine leishmaniasis in the targeted regions.

Materials and methods

Study Area

The study was conducted in three provinces in eastern Algeria, as shown in Figure 1. The first one, Batna, covers approximately 12,028.24 km^2 and is situated in the Aurès Mountains at an altitude of 1,048 m above sea level. This region experiences a varied climate, ranging from semi-arid in the north to arid in the south. Summers are moderately hot and dry, whereas winters are cold and humid with occasional snowfall. The average annual precipitation is approximately 326 mm.

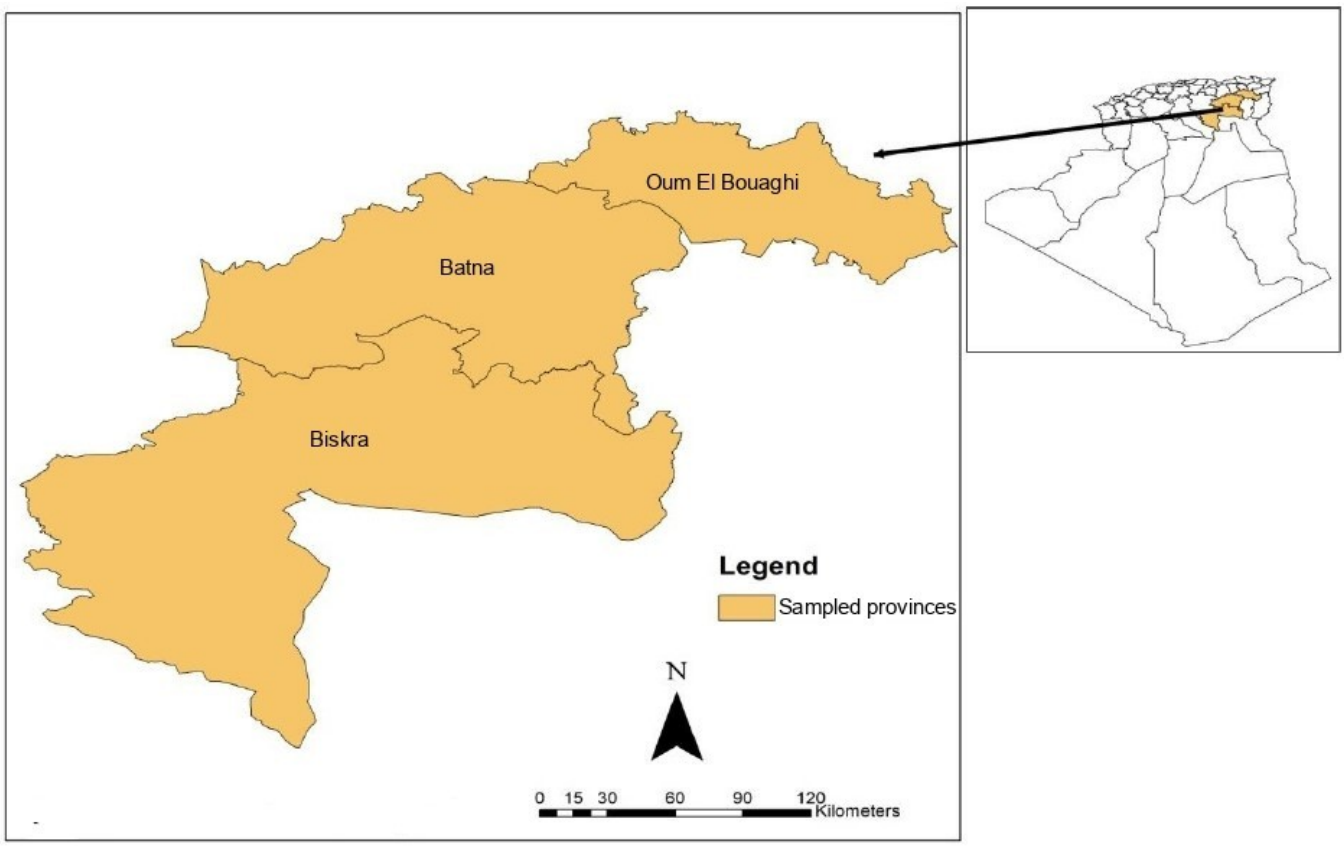

Figure 1. Geographical location of provinces sampled (Batna; Oum El Bouaghi, and Biskra).

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Oum El Bouaghi is the second province. It is located west of the Constantine plains and features three topographical domains: the northern Tell, the highlands, and the southern Saharan Atlas. The area includes vast plains and depressions forming endorheic basins, as well as mountainous regions ranging from 800 to 1,700 m in altitude, representing 25% of the total area. The climate is continental, with cold and wet winters and hot, dry summers.

The third province Biskra, spans 21,671.20 km^2 and lies at an altitude of 128 m. The northern part has a semi-arid to arid climate influenced by the Saharan Atlas, while the southern part has a typical Saharan climate (source: www.coordonnees-gps.fr).

Sample Collection and Serological Techniques

Between July 2021 and May 2024, a total of 421 dogs from veterinary clinics and rural areas were randomly selected for blood sampling: 285 from Batna, 100 from Oum El Bouaghi, and 36 from Biskra.

Approximately 5 ml of blood was collected by cephalic or radial vein puncture into dry tubes for serological analysis and into EDTA tubes for future molecular biology studies, with informed consent from the owners. Additional data were recorded for each animal, including geographic location, age, sex, breed, environment, nutrition, lifestyle, and use of preventive treatments (e.g., insecticides, anthelmintics). Dogs were also examined for clinical signs such as weight loss, lymphadenomegaly, emaciation, onychogryphosis, ocular or articular lesions, and cutaneous abnormalities.

Serological testing was performed on 347 dogs. Blood samples were centrifuged at 2,500 rpm for 4 minutes to separate the serum, which was stored at −20 °C until analysis. Serological testing was conducted using the commercial ELISA kit ID Screen® Leishmaniasis Indirect Test (VET-Innovate ID Diagnostics, France), following the manufacturer's protocol. The sensitivity and specificity (95% and 100% respectively) of this kit were demonstrated by Solano-Gallego et al., 2014. Negative and positive controls were tested in duplicate on each microplate. Optical density (OD) readings were taken at 450 nm using a microplate reader (Model PR 4100, Bio-Rad, Hercules, California). Washing steps were performed on PW40 microplate washer (Bio-Rad, Hercules, California).

The test was considered valid if the mean OD of the positive control exceeded 0.350 and the positive-to-negative control ratio was greater than three. Samples were interpreted as negative (<40%), doubtful (40–50%), or positive (≥50%) for *Leishmania infantum* antibodies.

In May 2024, a structured, face-to-face questionnaire was administered to the owners of seropositive dogs. Questions addressed their knowledge of canine leishmaniasis and the preventive strategies they used.

Statistical Analysis

A simple random sample comprising 347 dogs of different breeds, age categories, and both sexes, either apparently healthy or showing clinical signs consistent with leishmaniasis, was included in the study.

Descriptive statistical analyses were performed using Microsoft Excel 2016. Variables considered included sex, age group, lifestyle, clinical status, living environment, and province.

Univariate analysis was first conducted using the chi-square (χ^2) test to evaluate the association between each independent variable and *Leishmania* seropositivity. To assess the influence of intrinsic factors (age, sex, breed) and extrinsic factors (lifestyle, environment, intended use, province) on the seroprevalence of canine leishmaniasis, a logistic regression model was applied, following the methodology described by Saidani et al. (2024), and Saidani (2025).

The dependent binary variable was seropositivity, defined as the presence or absence of anti-*Leishmania* antibodies. Statistical analyses were conducted using R software version 4.4.3 (R Core Team, 2025). The optimal model was identified using the step function, which selects the best-fitting model based on Akaike Information Criterion (AIC). The most relevant explanatory variables retained in the final model were age category, preventive measures, environment (urban vs. rural), clinical status (symptomatic or asymptomatic), and province of origin. Spatial maps were generated using ArcGIS Desktop (version 10.8; ESRI, Redlands, CA, USA).

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Results

Descriptive analysis of the study population

347 dogs were sampled across East Algeria. Most of them were from rural areas 75.5% (262 dogs), while 24.5% (85 dogs) were from urban regions. A significant portion of the dogs (67.4%) were born in Batna.

The majority of dogs 60.8% (211 dogs) were guard dogs, while 21.3% (74 dogs) are used for other activities such as reproduction and companionship, and 17.8% (62 dogs) are hunting dogs. Males made up 62.2% of the population, while females constituted 37.1%.

The dogs were classified into four age groups, with the largest group consisting of those less than one year old, 35.7% (124 dogs). The other groups comprised 87 dogs between one and two years old, 68 dogs between two and three years old, and 68 dogs older than three years.

Positive Dog owners' perception on CanL and use of preventive measures

In our survey, Batna was found to be home to 50% of the positive dog owners questioned, which corresponds to 18 out of 36 total respondents. Oum El Bouaghi had 47.2% of the positive dog owners, translating to 17 out of 36 respondents. Biskra was notably lower, with only 2.7% represented, accounting for just one out of 36 respondents. Additionally, the majority of those surveyed had a medium level of education (58%). A significant majority, specifically 34 out of 36 owners (94.4%), displayed no previous knowledge about Canine Leishmaniasis (CanL). Despite this, 58.3% of them reported using general antiparasitic products to safeguard their dogs from other types of arthropods.

Interestingly, 47.2% of the owners believed that their dogs did not have any risk to contracting CanL. Furthermore, 50% estimated beliving that their dogs were at a risk of 5% for contracting CanL, while a small fraction, only 2.8%, thought the risk was as high as 10% (Figure 2) (Table I).

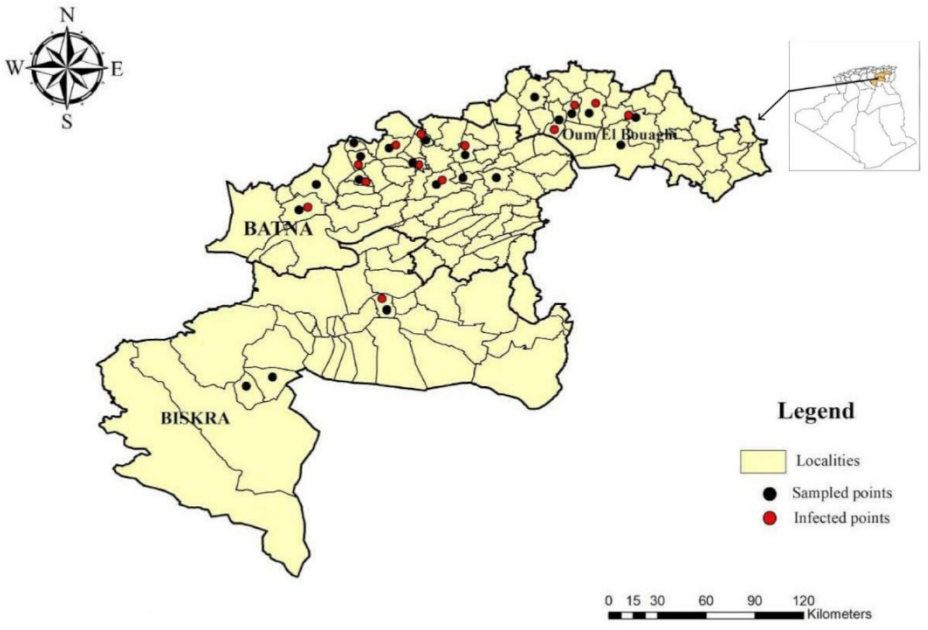

Figure 2. Geographic location of sampled localities and infected regions.

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Question		No. replies (%)
Education level	Low	6 (16.7%)
	Medium	21 (58.3%)
	Accepted	4 (11.1%)
	Heigh	5 (8.8%)
District of origin	Batna	18 (50%)
	Biskra	1 (2.7%)
	Oum El Bouaghi	17 (47.2%)
Have you ever heard of CanL?	Yes	2(5.5%)
	No	34(94.4%)
In your opinion, how great is the risk of any of your dogs having CanL throughout their lives	0%	17(47.2%)
	5%	18(50%)
	10%	1(2.8%)
Do you know of any measures to protect your dogs against CanL?	Yes	2(5.5%)
	No	34 (94.4%)
Do you use any measure to protect your dogs against CanL?	Yes	2(5.5%)
	No	36 (100%)
Which method do you use (ticks, fleas, etc.) ?	Collar	/
	General antiparasitic	21 (58.3%)
	Others	14(39%)

Table I. The questionnaire results from owners of seropositive dogs regarding their knowledge of canine leishmaniosis and the preventive methods used to prevent the infection.

CanL serological study results

Among the 347 dogs analysed, 10.37% (36 dogs) tested seropositive by ELISA. In Batna, the prevalence reached 50% with 18 positive cases, while in Oum El Bouaghi it was 47.22%. In contrast, Biskra showed a much lower prevalence of 2.8%, with only one positive case detected. By region, 18 out of 234 dogs (7.6%) were positive in Batna, 17 out of 83 dogs (20.4%) in Oum El Bouaghi and 1 out of 30 dogs (3.33%) in Biskra (Table II).

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Locality	No. Analysed points	No. analysed dogs (No. positive dogs)	Seropositivedogs (%)
-Batna :			
-Batna center	4	/	/
-Ras El Aïoun	22	1	4.5%
-N'Gaous	15	2	13.3%
-Talkhamt	5	2	40%
-Barika	88	4	4.5%
-Oued Chaaba	21	/	/
-Auyoun El Assafir	13	/	/
-El Hassi	17	4	23.5%
-Merouana	16	2	12.5%
-Djezzar	4	/	/
-Seriana	21	3	14.2%
-Guigba	8	/	/
Total :	234	18	7.6%
Biskra :			
-Biskra center	13	1	7.6%
-Sidi khaled	8	/	/
-Ouled Djellal	9	/	/
Total :	30	1	3.33%
Oum El Bouaghi :			
-Oum El Bouaghi center	17	7	41.1%
-Aïn Fekroun	39	7	17.9%
-HanchirToumghani	9	1	11.1%
-Aïn Kercha	9	2	22.2%
-Aïn Zitoun	4	/	25%
-Aïn M'lila	5	/	/
Total :	83	17	20.4%
Total	347	36	10.37%

Table II. Seroprevalence of canine Leishmania infantum infection observed in each locality.

Clinical signs	Seropositive	(%) of symptomatic positive dogs (N/12)
Cutaneous lesions	6	50,00%
Onychogryphosis	4	33,33%
Lymphadenomegaly	2	16,67%
Emaciation	8	66,67%
Ocular lesions	2	16,67%

Table III. Results of canine Leishmania infantum seroprevalence in dogs with clinical signs.

Twelve out of 36 (33.3%) seropositive dogs were considered symptomatic. Several clinical signs were observed in

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seropositive dogs, including cutaneous lesions in 50% (6/12), emaciation in 66.6 % (8/12), lymphadenomegaly in 16.6% (2/12), onychogryphosis in 33.3% (4/12), and ocular lesions in 16.6% (2/12) (Table III).

Significant results ($p \leq 0.05$) were observed for several factors associated with the seropositivity of canine leishmaniasis in our study, according to the Chi-square test. These factors include age, breed, activity, clinical status, area, feed, province and lifestyle. However, preventive measures, and sex did not show significant associations ($p > 0.05$).

Logistic regression analysis revealed that age was associated with seropositivity with a significant results ($p < 0.05$) and older dogs (>3 years) were significantly higher risk of infection compared to dogs under one year of age [OR = 4.26, 95% CI: 1.45–13.76; p = 0.01].

Province and area were strongly associated with seropositivity ($p < 0.01$). Conversely, urban areas were associated with a lower risk of infection [OR = 0.033, 95% CI: 0.0015–0.225; p = 0.003]. Additionally, seroprevalence varied by province, with dogs from the Oum El Bouaghi region showing a significantly higher risk of infection [OR = 3.54, 95% CI: 1.59–8.05; p = 0.002].

Clinical status also showed a very strong association ($p < 0.001$), dogs with indicative symptoms for canine leishmaniasis were highly associated with a positive serology [OR = 4.56, 95% CI: 1.844–11.22; p = 0.0008].

That means all these variables are a definitive predictors of L. infantum infection in exposed dogs according to our results. Results of the analysis are summarized (Table V and VI).

Factors	Levels	No. Dogs analysed	No. Seropositive dogs (%)	p value
Sex	Male	218	23 (10.5)	0,889
	Female	129	13 (10.1)	
Age	<1 year	124	6 (4.8)	0,024*
	1-2 years	87	8 (9.2)	
	2-3 years	68	9 (13.2)	
	>3 years	68	13 (19.1)	
Breed	Local	149	21 (14.1)	0,049*
	Purebred	198	15 (7.6)	
Activity	Guard	211	27 (12.8)	0,016*
	Hunting	62	8 (12.8)	
	Other	74	1 (1.3)	
Clinic	Symptomatic	55	12 (21.8)	0,002*
	Asymptomatic	292	24 (8.2)	
Area	Rural	262	35 (13.3)	0,001*
	Urbain	85	1 (1.2)	
Feed	Yes	74	1 (1.3)	0,004
	No	273	35 (12.8)	
Province	Batna	234	18 (7.7)	0.0019*
	Biskra	30	1 (3.3)	
	OEB	83	17 (20.5)	
Lifestyle	Chained	240	18 (7.5)	0.008*
	Unchained	107	18 (16.8)	
Prevention measures	Yes	92	5 (5.4)	0,069
	No	255	31 (12.1)	

Table IV. Seroprevalence of canine leishmaniasis in relation to different variables using the Chi-square test. *The result is significant for Chi-square test at $p \leq 0.05$.

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Factor and levels		Estimate	OR	p value	95% CI for OR
Age	<1 year				
	1-2 years	0.6254	1.868	0.290	0.587- 6.225
	2-3 years	1.1151	3.049	0.0554	0.991- 10.078
	>3 years	1.4516	4.269	0.01011 *	1.459- 13.761
Prevention mesasures	Yes				
	No	1.1959	3.3065	0.059	0.878- 11.019
Province	Batna				
	Oum El Bouaghi	1.2669	3.549	0.002**	1.596 -8.053
	Biskra	-1.6945	0.183	0.14138	0.008 -1.191
Clinical examination	Asymptomatic				
	Symptomatic	1.5193	4.569	0.0008 ***	1.844 -11.227
Environment	Rural				
	Urban	-3.4096	0.033	0.003 **	0.001- 0.225

Table V. Results of the logistic regression. *The result is significant for p ˂ 0.05.

Discussion

This study provides a recent report of *Leishmania infantum* seroprevalence in three provinces in eastern Algeria. Notably, this marks the first instance of such investigations being conducted in these regions.The results of this study showed the overall seropositivity of 10.37% in canine across these three localities. This prevalence was estimated by the ELISA test which remains one of the most frequently used serological methods and is recommended by the World Organization for Animal Health for performing canine leishmaniasis surveillance studies and to detect prevalence of infection (Solano-Gallego et al., 2014). ELISA is easy to perform and interpret, being particularly useful in field study settings, where a large number of samples must be analysed (Maurelli et al., 2020).

Our results remain lower than those of other studies carried out in Algeria: 68% by Bia et al. (2022), 36% by Medkour et al. (2020), 18% by Bellatreche et al. (2021). In neighbouring countries, recently recorded prevalences vary between 58% and 66% in Tunisia by Mhadhbi et al. (2021) and Bouattour et al. (2021) and 33% in Morocco by Idrissi et al. (2021).Our results showed a prevalence of around 46.7% in asymptomatic dogs, which demonstrates the importance of serological diagnosis in prevention, given that these carriers remain neglected.Therefore, a fast, sensitive, and specific tool for detecting *L. infantum* infection in dogs would be highly beneficial, enabling effective control measures in regions where the human infection is frequent.

The prevalence of leishmaniasis in OEB is higher in dogs compared to the two other sampled localities, with Batna being the second most affected region. This is likely due to the presence of *Phlebotomus perniciosus*, particularly in Ain Fekroun and Hanchir Toumghani (two regions in Oum El Bouaghi known for their abundance of sand flies). These findings indicate that leishmaniasis remains a significant public health concern in these regions (Kabbout et al., 2015).

With regard to the influence of the risk factors discussed in our study, we found that infestation increases overall as the animals get older, from 4.8% (under 1 year of age) to 19% (over 3 years of age) with results statistically significant. Our results are in line with those reported by Lopes et al. (2024), who reported a clear correlation between age and frequency of infection, underlining the need for targeted surveillance and preventive measures for older dogs, who are at greater risk.

Similar findings were also reported by Chiyo et al. (2023), who explained their results by the exposure to the vector and the humoral response, which may take several months to develop.In other studies on this subject, the research suggests that age-related immunity changes determine a dog's response to *Leishmania*, with older dogs exhibiting stronger resistance.

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Regarding sex, we did not observe significant differences between males and females. Our findings are consistent with those of previous studies, in which this association is considered weak (Belo et al., 2013; Miranda et al., 2008).Although some studies have reported a higher prevalence in males, this has been attributed to the greater roaming behavior of male dogs (Barbosa et al., 2022; Leontides et al., 2002; Penaforte et al., 2013) or to differences in host immune responses (Maia & Campino, 2018).

In regard to breed, statistical analysis revealed significant differences between the local dogs and other purebreds. Some authors point out that there is no significant difference in the prevalence of leishmaniasis between dog breeds (Amusategui et al., 2003; Oliveira et al., 2016), while others report varying levels of susceptibility, with some breeds showing higher or lower infection rates. It should be noted that the impact of the breed is also influenced by the environment in which the dogs live and their type of activity.

In this survey, the living environment was identified as a risk factor, with dogs in rural areas showing higher exposure to infection. This aligns with findings from similar studies and may be due to increased contact with wild animals and disease vectors, as transmission can also happen in natural environments (Feliciangeli, 2004). Even with the urban spread of leishmaniasis, rural traits remain (Escobar et al., 2018). Studies indicate that animal husbandry impacts local health by generating organic waste, which attracts and supports the vector's presence (Barata et al., 2005). Environmental features such as tall grass, green spaces, and organic matter have also been associated with the persistence of the disease (Campino & Maia, 2013).

Dogs' activities appear to influence infestation rates, with guard and hunting dogs showing higher levels of infestation than other groups. These observations can be explained by the fact that these types of dogs are more accessible to sandflies and therefore more exposed to infection by *Leishmania infantum*. Our results corroborate those found by Oliveira et al. (2021) who reported greater infestation in hunting and guard dogs due to the environment in which these animals are kept, since many of these dogs live adjacent to the feeding and breeding sites of sandflies.Moreover, these animals are exposed to forest areas during hunting activities, usually in twilight and post-twilight periods, coinciding with peak sandfly activity (Lago et al., 2020; Silva et al., 2018; Trájer et al., 2018).

Our findings regarding the lifestyle of dogs indicated a higher rate of infestation among unchained animals. This can be attributed to their increased exposure to vectors compared to dogs that are kept restrained. These results are consistent with those of Barbosa et al. (2022), who reported that dogs living in peridomestic environments or with free access to the streets were 1.5 times more likely to become infected. Similarly, Belo et al. (2013) found that the likelihood of *Leishmania* infection was lower in dogs confined to domestic areas.

In our study, well-fed dogs were significantly less infested than poorly nourished ones, with the frequency of infestation being twelve times lower. This finding aligns with research conducted in the Mymensingh district of Bangladesh, where dogs in poor health were found to be approximately 13 times more susceptible to *Leishmaniadonovani* infection compared to healthy dogs (Islam et al., 2017).

Similarly, a study conducted in Brazil reported a negative relationship between nutritional status and leishmaniasis in dogs, indicating that better nutrition is associated with a lower risk of infection, and vice versa. Thus, the nutritional status of the host plays a crucial role in the defense against pathogens. Undernutrition can lead to a compromised immune system, increasing the risk of both asymptomatic infections and severe diseases. It contributes to heightened host susceptibility and worsened pathophysiological outcomes through multiple biological pathways (Nweze et al., 2020).

From the 36 seropositive dogs, only 12 (33.3%) showed clinical signs compatible with CanL. It is also known that, in endemic areas, a small proportion of dogs display symptoms of CanL. This is in agreement with others (Bouattour et al., 2021; Vélez et al., 2019). This result suggests that there is the possibility that some dogs are in an early stage of infection (Fisa et al., 2001; Miró et al., 2012) or are immunologically resistant and only transiently seropositive. Therefore, it's crucial to treat these dogs to reduce their parasite load, without neglecting the role of asymptomatic dogs.

The survey conducted among dog owners revealed a general lack of awareness regarding *Leishmania infantum* infestation, with the majority of respondents uninformed about the risk to their animals. Therefore greater attention should be paid to this vector-borne infectious disease, and interventions should prioritize health education for dog owners and the development of programs focused on the prevention and control of zoonotic diseases.

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Conclusion

The results obtained in our study highlight a significant seroprevalence of canine leishmaniasis in the population studied, reflecting active circulation of *Leishmania infantum* in eastern Algeria. These results confirm the central role of the dog as the main reservoir of the pathogen and underline the importance of an integrated surveillance approach, combining early diagnosis, epidemiological monitoring and targeted prevention measures as well as raising awareness among veterinary professionals and owners to improve management of this parasitosis.

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The authors sincerely thank owners for their participation and trust, as well as veterinarians and field professionals whose collaboration and commitment were essential to the successful completion of this work. Their support in sampling,clinical examination,and data collection was invaluable.

This study was carried out within the framework of a doctoral research project in veterinary sciences.

Ethical approval

In accordance with national legislation (Ordinance No. 88-08 of January 26, 1988, relating to veterinary medicine and animal health protection), the local ethics committee authorized the study, including field collection and handling of animals.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

Conceptualization: S.B; S.R; H.Z;R.S; Methodology: H.Z; S.B; R.S; Formal analysis: S.B; K.S; S.R; D.E.G; Investigation: H.Z; S.B; S.R; R.S; Writing original draft preparation: S.B; F.Z; K.S; S.R; R.S; Writing, review and editing: S.B; F.Z; K.S; S.R; A.B; D.E.G; R.S; Visualization: S.B; S.R; R.S; Supervision: S.R; R.S.

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All data available upon request to the corresponding author.

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