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Paper

Leishmaniasis Awareness and Screening Among Dog Owners in Tiaret, Algeria

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Abstract

Leishmaniases are zoonotic diseases, and the risk of human transmission of leishmaniasis caused by*Leishmania infantum* in endemic areas is increasing public health concerns.This study sought to determine the knowledge of dog owners in the province of Tiaret regarding Human Visceral Leishmaniasis (HVL) and Canine Visceral Leishmaniasis (CVL), and to carry out serological detection of HVL, using a variety of serological diagnostic methods, including enzyme immunosorbent assay (ELISA), indirect fluorescent antibody test (IFAT), immunochromatographic test (IT-LEISH), and Western Blot (WB), this study sought to determine the knowledge of dog owners in Tiaret Province regarding Human Visceral Leishmaniasis (HVL) and Canine Visceral Leishmaniasis (CVL). To establish the risk between owning a *Leishmania* positive dog and the human infection, serological screening of the owners' dogs using IFAT was carried out. Every single participant, who ranged in age from 21 to 66, was male. The majority were farmers and ignorant. A questionnaire regarding HVL and CVL was completed by each of them. Only a small percentage of respondents accurately completed the questionnaire; most were unaware of these illnesses, and some mistook them for other diseases. Additionally, the existence of doubtful cases (3%) by IFAT was discovered during the serological screening of dog owners for HVL. Finally, five of the nine people whose dogs tested positive and who were subjected to the Western Blot method were found to be positive. Finally, we can highlight the ignorance of leishmaniasis among dog owners, and the importance of making these people aware of the risks of this disease for dogs and humans.

Keywords

knowledge, Human Visceral Leishmaniasis, Canine Visceral Leishmaniasis, dog owners, serology

Introduction

Leishmaniasis is a parasitic disease caused by intracellular parasites of the genus *Leishmania*. It is transmitted to humans through the bite of sandflies of the genus *Phlebotomus* in the Old World and *Lutzomyia* in the New World. At least 20 species of *Leishmania* can infect humans. The disease is endemic in Asia, Africa, the Americas, and the Mediterranean region. Globally, 1.5 to 2 million new cases are reported each year, with approximately 350 million people at risk of infection, and an estimated 70,000 deaths annually (Torres-Guerrero et al., 2017).

In Algeria, leishmaniasis manifests primarily as visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL). In the northern part of the country, CL is caused by *Leishmania infantum* (L. infantum) MON-24, transmitted by *Ph. perfiliewi* (Izri and Belazzoug, 1993), while VL is caused by *L. infantum* MON-1, with *Ph. perniciosus* as the confirmed vector (Harrat et al., 1996). Canids are proven reservoirs for both strains (Benikhlef et al., 2004; Bessad et al., 2012).

In the southern and steppe regions, CL is mainly caused by *L. major* MON-25, transmitted by *Ph. papatasi* (Harrat et al., 1996; Izri et al., 1992). The wild rodents *Psammomys obesus* and *Meriones shawi* are known reservoirs (Belazzoug, 1983; 1986). More recently, *L. killicki*, part of the *L. tropica* complex, has been reported in both southern and northern Algeria (Harrat et al., 2009), transmitted by *Ph. sergenti* (Boubidi et al., 2011). While *Ctenodactylus gundi* is a confirmed reservoir in Tunisia (Jaouadi et al., 2011), in Algeria *Massouteira mzabi* is considered a suspected reservoir (Boubidi et al., 2011).

Visceral leishmaniasis cases in Algeria are mainly concentrated in the northern and central regions, such as the Hauts Plateaux, as well as in some steppe areas. A study covering the period from 1998 to 2008 reported an average of 142 VL cases per year, corresponding to an annual incidence rate of 0.45 cases per 100,000 inhabitants (Adel Amel, 2014).

In Tiaret, there has been a noticeable increase in the prevalence of canine leishmaniosis, driven by factors such as urbanization, environmental changes, and the movement of infected animals. The disease affects not only canine health but also poses a significant risk to human health, highlighting the close connection between animal and human health (Bia et al., 2022). The presence of infected dogs in the study area increases the likelihood of transmission of *L. infantum*—the causative agent of both canine and human VL—from dogs, the primary reservoir, to humans via the bite of infected female sandflies (Portela et al., 2019; Bia et al., 2022; Dias et al., 2023). This represents a potential health threat to dog owners.

In this context, the objectives of the present study were to assess dog owners' knowledge of human and canine visceral leishmaniasis (HVL and CVL), to perform serological screening of HVL in dog owners using several diagnostic techniques (IT-LEISH, IFAT, ELISA, and Western Blot), and to identify potential risk factors—particularly the risk of owning a *Leishmania*-positive dog.

Materials and Methods

The survey was conducted in the Wilaya of Tiaret (35°22′15″ N, 1°19′01″ E), an agricultural region characterized by a heterogeneous landscape comprising mountainous areas in the north, high plains in the center, and semi-arid regions in the south. The climate is marked by two distinct seasons: a harsh winter and a hot, dry summer. Annual precipitation ranges from 300 to 400 mm, distributed relatively evenly throughout the year (Mohammed and Benchaben, 2016).

Questionnaire

A questionnaire-based survey was conducted among dog owners from various districts across the northern, central, and southern regions of the Wilaya. Data collection began after obtaining oral consent from each participant. The questionnaire was written in Arabic, using simple and accessible language, and any unfamiliar or difficult terms were explained by the investigators.

A total of 80 male participants, aged between 21 and 66 years and representing diverse educational backgrounds and occupations, took part in the study. The questionnaire collected personal information, assessed their knowledge of human and canine visceral leishmaniasis (HVL and CVL), and gathered details regarding their dogs. All responses were recorded on clinical data sheets.

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Samples collection

Humans (dog owners)

Blood samples were collected from each owner's dog, resulting in a total of 80 samples. A volume of 5 mL of blood was collected from each dog owner into dry tubes by nurses from the local public health establishments (EPSP) in each locality. After centrifugation, the sera were stored at −20 °C and subsequently sent to the Parasitology and Mycology Laboratory at the Centre Hospitalo-Universitaire Mustapha Bacha in Algiers (Algeria) for the IT-LEISH immunochromatographic test and Western Blot (WB) analysis.

The Indirect Fluorescent Antibody Test (IFAT) and Enzyme-Linked Immunosorbent Assay (ELISA) were performed at the WHO Collaborating Centre for Leishmaniasis, National Microbiology Centre, Instituto de Salud Carlos III, Madrid, Spain.

Animals (dogs)

For each dog, 4 mL of peripheral blood were drawn into dry tubes. After serum separation, a total of 213 samples were stored at −20 °C and subsequently transferred to the WHO Collaborating Centre for Leishmaniasis, National Centre for Microbiology, Instituto de Salud Carlos III (Madrid, Spain) for analysis using the Indirect Fluorescent Antibody Test (IFAT).

Serological tests

Fluorescent Antibody Test (IFAT)

To detect anti-*Leishmania* antibodies, *Leishmania infantum* promastigotes (strain MHOM/FR/78/LEM75) were used as antigens for the Indirect Fluorescent Antibody Test (IFAT), following the protocol described by Alvar et al. (2004). Secondary antibodies—fluorescein isothiocyanate (FITC)-conjugated anti-dog IgG or anti-human IgG (whole molecule)—were obtained from Sigma-Aldrich (USA) and appropriately diluted in 0.01% Evans Blue.

Phosphate-buffered saline (PBS), pH 7.2, was used to dilute the sera at serial dilutions of 1:20, 1:40, 1:80, and 1:160. The positivity threshold was set at 1:80 for dogs and 1:40 for humans.

Enzyme-Linked Immunosorbent Assay (ELISA)

According to the protocol of Alvar et al. (2004), an in-house ELISA was performed using soluble *Leishmania* antigens. Optical density was measured at 493–620 nm using a spectrophotometer on a Mercedes microplate reader. Samples with absorbance values above 0.200 were considered positive, while those below 0.200 were considered negative, based on the established cut-off value.

Immunochromatographic test (IT-LEISH)

The immunochromatographic test known as IT-LEISH uses a strip coated with the recombinant rk39 antigen. The test was performed following the instructions provided in the user manual (IT-LEISH, Bio-Rad, France).

Western Blot

Western blot analysis was performed only for dog owners who tested positive by IFAT. The procedure was conducted using *Leishmania infantum* WB IgG kits (Leishmania IgG Western Blot, LDBIO Diagnostics, France).

Statistical analysis

Excel Stat 2016 was used to calculate Cronbach's alpha coefficient and assess the reliability of the questionnaire responses. This coefficient is a well-established indicator of the internal consistency and reliability of questionnaire items. According to Cronbach (1951), a Cronbach’s alpha value greater than 0.70 indicates good consistency.

Percentages were compared using a chi-square test with a significance threshold set at $p < 0.001$.

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Results

Demographic characteristics of questioned dog owners

Characteristics	Number (%)
Sex	
Men	80(100)
Age (years)	37,58 ± 10,77 [21-66][a]
Profession	
Farmer	63(78,75)
Public servant	11(13,75)
Unemployed	6(7,5)
Academic level	
Average level	12(15)
Elementary level	14(17,5)
University level	7(8,75)
Illiterate	47(58,75)
Dog owner's origin	
Ain Deheb	1(1,25)
Ain Kermes	10(12,5)
Frenda	39(48,75)
Hamadia	1(1,25)
KsarChellala	13(16,25)
Medroussa	1(1,25)
Meghila	3(3,75)
OuedLili	1(1,25)
Rahouia	2(2,5)
Tiaret chief town	9(11,25)
Habitat type	
Rural	62(77,5)
Suburban	3(3,89)
Urban	12(15,58)
Rural, urban	3(3,89)
Animal husbandry	
No	11(14,28)
Yes	66(85,71)
Frequent veterinary visit	
No	70(87,5)
Yes	10(12,5)

Table I. Demographic characteristics of the surveyed dog owners (N = 80). *a* Mean ± standard deviation [Minimum; Maximum].

A total of 80 dog owners (100%) participated in the study and completed the questionnaire. All participants were male, with a mean age of 37.58 ± 10.77 years (range: 21–66), and represented various occupational backgrounds. The

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majority were farmers (78.75%), followed by civil servants (13.75%) and unemployed individuals (7.5%).

In terms of educational level, the largest proportion of dog owners were illiterate (58.75%), followed by those with elementary (17.5%) and secondary (15%) education; only 8.75% had received university-level education.

Participants were distributed across the northern, central, and southern regions of the Tiaret province. Frenda accounted for the highest proportion (48.75%), followed by Ksar Chellala (16.25%), Ain Kermes (12.5%), and Tiaret Chief Town (11.25%). Smaller proportions were reported in Meghila (3.75%), Rahouia (2.5%), Ain Deheb, Hamadia, Medroussa, and Oued Lili (each 1.25%).

Most dog owners (77.5%) resided in rural areas, with a smaller percentage in urban areas (15.58%) and a minority in peri-urban or mixed settings (3.89%). Additionally, 85.71% of owners were involved in animal husbandry.

With regard to veterinary care, the majority of dog owners (87.5%) reported not attending regular veterinary visits. Detailed demographic data are presented in Table I.

Knowledge of dog owners about HVL and CVL

To assess the knowledge of human and canine visceral leishmaniasis (HVL and CVL), participants completed a questionnaire consisting of nine questions: five related to HVL and four to CVL (Table II).

The responses to the questions “What is HVL?” and “What are the signs of HVL?” were identical. The majority of participants (87.5%) were unaware of HVL and its symptoms, while only 6.25% demonstrated awareness of the disease. Only 9 dog owners (11.25%) knew that HVL is a curable condition. Although there were no incorrect answers, more than half of the respondents (88.75%) chose not to respond to this item.

Furthermore, 90% of dog owners were unfamiliar with the disease and its symptoms, and only 8.75% provided correct answers. Regarding the final question, which concerned the mode of transmission of CVL, 1.25% of participants answered incorrectly, 6.25% correctly, and the vast majority (92.5%) did not know the answer.

These findings reveal a low level of knowledge among dog owners, with a notable correlation between their understanding of HVL and CVL. High chi-square values and low p-values indicate a statistically significant association between the responses and awareness of these diseases.

Additionally, the heat map (Figure 1) visualizing respondents’ knowledge about HVL and CVL shows that most participants lacked knowledge, a small number provided incorrect information, and only a minority answered correctly.

The questionnaire showed excellent internal consistency, with a Cronbach’s alpha coefficient of 0.914, indicating a high level of reliability and strong correlation among the questionnaire items.

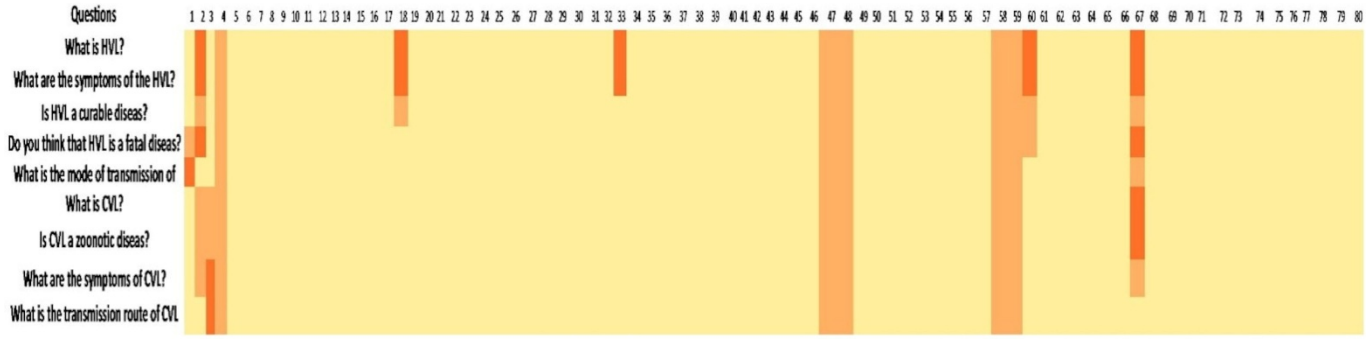

Figure 1. Heat map illustrating the knowledge of dog owners regarding human and canine visceral leishmaniasis.

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Questions	*Wrong (%)*	*Correct (%)*	*Don't know (%)*	*Khi², P value*
What is HVL?	5 (6,25)	5 (6,25)	70 (87,5)	Khi²=158,23 P<0,0001
What are the symptoms of the HVL?	5 (6,25)	5 (6,25)	70 (87,5)	Khi²=158,23 P<0,0001
Is HVL a curable disease?	0	9 (11,25)	71 (88,75)	Khi²=96,1 P<0,0001
Do you think that HVL is a fatal disease?	2 (2,5)	7 (8,75)	71 (88,75)	Khi²=166,53 P<0,0001
What is the mode of transmission of HVL?	1 (1,25)	6 (7,5)	73 (91,25)	Khi²=181,83 P<0,0001
What is CVL?	1 (1,25)	7 (8,75)	72 (90)	Khi²=174,41 P<0,0001
Is CVL a zoonotic disease?	1 (1,25)	7 (8,75)	72 (90)	Khi²=174,41 P<0,0001
What are the symptoms of CVL?	1 (1,25)	7 (8,75)	72 (90)	Khi²=174,41 P<0,0001
What is the transmission route of CVL to dogs?	1 (1,25)	5 (6,25)	74 (92,5)	Khi²=189,48 P<0,0001

Table II. Knowledge of the dog owners regarding HVL and CVL (N= 80).

Serological results of dog owners

The prevalence of human visceral leishmaniasis, as determined by three serological tests—IT-LEISH, ELISA, and IFAT—is shown in Figure 2. No positive cases were detected by either ELISA or IT-LEISH. However, IFAT identified 3% of cases as indeterminate.

Serological results of dogs

Out of the total 213 dogs tested, 20 showed a positive result by IFAT, corresponding to a prevalence of 9% (Figure 3).

Western Blot results

Among the dog owners, nine had at least one dog that tested positive. These individuals were further tested by Western Blot, and five were confirmed positive. Two of the owners had dogs with borderline IFAT results at a 1:40 dilution; however, these were negative by Western Blot.

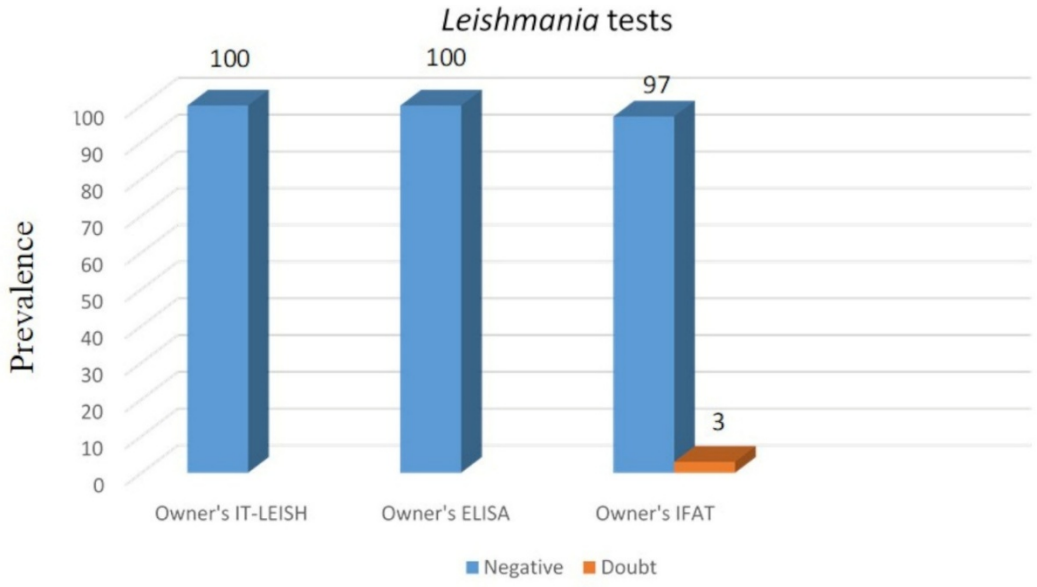

Figure 2. Histogram showing the prevalence of human visceral leishmaniasis using three different serological tests (N=80).

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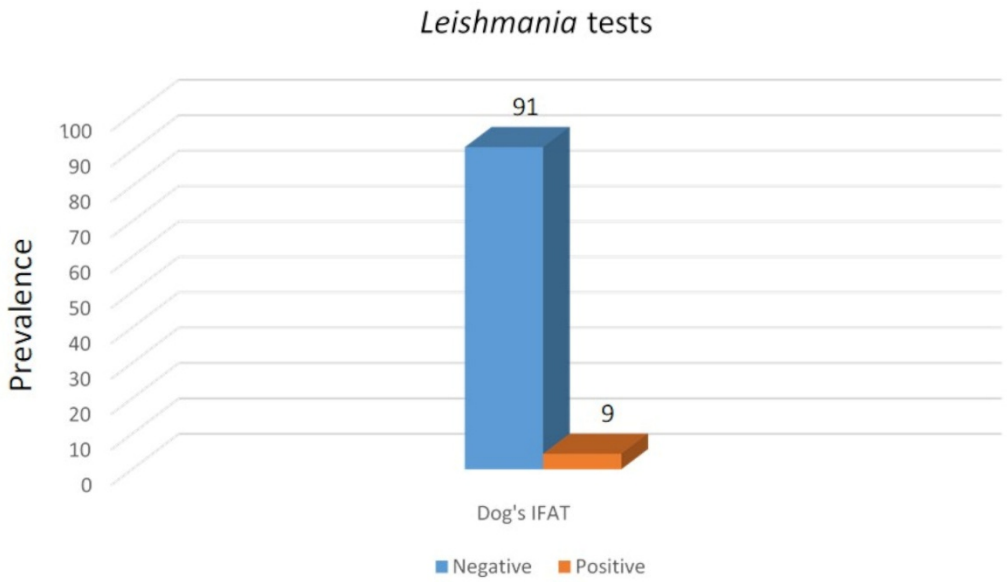

Figure 3. Histogram showing the prevalence of canine visceral leishmaniasis (N=213).

Discussion

In our survey, all participating dog owners were men (80/80), aged between 21 and 66 years, with the majority residing in the central region of Tiaret province, particularly in Frenda (48.75%). Most of the participants were uneducated (58.75%), worked as farmers (78.75%), and lived in rural areas (77.5%) where animal husbandry was also common (85.71%). These socioeconomic and environmental factors explain the prevalent use of dogs for work-related activities and security purposes (Bia et al., 2022).

The survey also revealed that the majority of dog owners lacked knowledge about HVL and CVL. Specifically, 87.5% and 90% of respondents, respectively, were unaware of these diseases, while only 6.25% and 8.75% provided correct responses regarding severity, symptoms, and transmission mechanisms. Owners who gave correct answers were generally those previously informed by veterinarians or who had personal experience with canine leishmaniasis.

These findings are consistent with those of Khbou et al. (2019), who reported that less than 50% of surveyed owners were able to define leishmaniasis accurately, except for medical and veterinary professionals or those with direct experience. Similarly, Berhe et al. (2018) found that fewer than 50% of participants in Ethiopia's Welkait district had knowledge of HVL. In contrast, Diriba and Gebremedhin (2023) reported much higher awareness levels in their study, with 84% of participants recognizing canine leishmaniasis, and Alemu et al. (2013) found that 87.4% were familiar with human visceral leishmaniasis.

Regarding HVL symptoms, only 6.25% of participants provided correct answers, and some (6.25%) confused cutaneous leishmaniasis with visceral leishmaniasis—a finding also noted by Khbou et al. (2019), who reported a 9% rate of misidentification. Additionally, 11.25% of owners believed HVL to be a curable disease, while 8.75% considered it fatal and untreatable. These proportions are lower than those reported by Alemu et al. (2013), Berhe et al. (2018), and Diriba and Gebremedhin (2023), who found that 88.26%, 86.4%, and 72% of participants, respectively, believed HVL to be curable.

Concerning the zoonotic transmission of CVL, 90% of dog owners were unaware of the risk to humans. Only 8.75% provided correct answers, compared to 67% in the study by Khbou et al. (2019) and 80.5% in Diriba and Gebremedhin (2023), where 76% also correctly identified the symptoms. Awareness of the vector was also low in our study (6.25%), whereas 33% of respondents in Diriba and Gebremedhin's study knew that sandflies transmit the disease.

Despite these knowledge gaps, the questionnaire showed high internal consistency, with a Cronbach's alpha of 0.914, indicating excellent reliability (Cronbach, 1951). A statistically significant association between responses and knowledge of HVL and CVL was supported by high chi-square values and $\mathbf{p < 0.001}$.

Four serological techniques—IT-LEISH, IFAT, ELISA, and Western Blot—were used to screen the owners. All were

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negative by IT-LEISH and ELISA, while IFAT returned negative results in 97% of cases, with 3% classified as indeterminate (1:40, the positivity threshold for humans). These doubtful results could be due to cross-reactivity with other parasites such as *Babesia*, *Anaplasma*, or *Trypanosoma*.

Western Blot, a highly sensitive and specific method for HVL serodiagnosis (Heidari et al., 2019), confirmed five positive cases among dog owners with at least one seropositive dog. This finding supports the hypothesis that owning a *Leishmania*-positive dog increases the risk of human infection, as dogs serve as parasite reservoirs and facilitate transmission via sandfly bites.

The discrepancy in serological results may be attributed to various factors, including the presence of CVL-positive dogs in the surrounding area (9% by IFAT), the endemic status of leishmaniasis in Tiaret (Bia et al., 2022), the shared pathogenetic agent of HVL and CVL (*L. infantum*) (Portela et al., 2019; Maia et al., 2023), rural living conditions, limited veterinary care, and the varying performance of diagnostic techniques (Silva et al., 2014; Freire et al., 2019; Ortalli et al., 2020; Soares et al., 2022; Ismail et al., 2023; Iatta et al., 2023).

Conclusion

The primary concern for dog owners regarding leishmaniasis is that their dogs are recognized reservoirs of the *Leishmania* parasite. Given the difficulty of treating leishmaniasis in dogs and the potential risk of transmission to humans, prevention remains the most effective strategy.

Based on the findings of this study, it is evident that the majority of dog owners are either completely unaware or insufficiently informed about both human and canine visceral leishmaniasis (HVL and CVL). Therefore, it is essential to improve their knowledge by providing accurate and reliable information through educational initiatives led by medical and veterinary professionals.

In addition, to monitor the health status of dogs and screen for potential infections in both animals and owners, it is important to encourage regular visits to veterinary clinics and promote the use of reliable diagnostic techniques.

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Author contributions

B.T, M.H.: writing the first draft, investigation; Z.H.: validation, supervision; C.S., J.N., and L.B.: helped with the study's conception and design; K.M., S.K.M., S.S.M., B.N.A.: writing, review, and editing; D.G.: statistical analysis; K.B. and O.A.M.: investigation; L.I. and J.M.: supervision, review, and editing; and all authors read and approved the final manuscript.

Ethics statement

This study was approved and authorized by the Scientific Committee of the Institute of Veterinary Sciences at Ibn Khaldoun University of Tiaret, Algeria (Approval No. 205/VRPG/2017). All participants provided written informed consent, and blood samples were collected at the hospital level in accordance with ethical guidelines.

Conflict of interest

The study's authors affirm that they have no conflicts of interest.

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