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Original Article

The Seroprevalence of Canine Leishmaniasis and a Retrospective Survey of Human Leishmaniasis in the Constantine Region of Algeria

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Abstract

Background: In Algeria, leishmaniasis is characterized by an endemic nature, which appears in humans in the two known forms: visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL). This study aimed to determine the seroprevalence of canine leishmaniasis within a group of dogs and the incidence of human leishmaniasis.

Methods: We performed a serological survey of canine leishmaniasis (CanL) in 84 asymptomatic dogs in Constantine wilaya (north-west of Algeria). The collected dog sera were tested by the indirect ELISA method. The study considered multiple risk factors, including the breed, sex, age, and location of the dogs. The dogs whose blood samples were taken during this survey came from the following municipalities in Constantine: El-Khroub, Ali Mendjeli, Zighoud Youcef, Aïn Smara, Hamma Bouziane, Didouche Mourad, and Constantine. In parallel, an epidemiological study of human leishmaniasis in the Constantine region was conducted. We collected retrospective data on the two forms of human leishmaniasis from Constantine's Department of Public Health between 2016 and 2024.

Results: The incidence of CanL observed in the study was 8.33% (with 7 dogs testing positive. During the nine-year study period, 136 cases of CL and VL were recorded. The predominance of CL (with 124 cases, 91.18%) over VL (12 cases, 8.82%) was statistically significant (χ^2=92.24; P<0.001) throughout the study duration.

Conclusion: The high seroprevalence of leishmaniasis in asymptomatic owned dogs, along with the significant frequency of human cases of CL and VL in the Constantine region, indicated that leishmaniasis remained a major public health problem in Algeria.

Introduction

Canine leishmaniasis (CanL) is a zoonosis caused mostly by the species *Leishmania infantum*, a protozoan of the Trypanosomatidae family, transmitted by phlebotomine sand flies (order Diptera) (1). The disease has been observed in several countries and areas, and it is endemic in the Mediterranean basin (including Algeria, Spain, southern France, Italy, Malta, Greece, Turkey, Egypt, Tunisia, Morocco, and others), South America (Brazil, Argentina, Uruguay, Paraguay, and Venezuela), and Asia (Iraq, Iran, the former Asian republics of the Soviet Union, Pakistan, and China) (2-4). CanL continues to be an issue for public and animal health, even in non-endemic countries (such as the United Kingdom and Germany), due to the importation of clinically diseased or asymptomatic dogs (5).

Like other Mediterranean countries, Algeria is recognized for the endemic occurrence of leishmaniasis. In a meta-analysis of 12 serological or molecular studies carried out in Algeria on animal leishmaniasis and published between 2004 and 2022, Khelifi-Touhami et al. observed the following overall prevalence: 21.2% in dogs, 25.3% in stray cats, 20.8% in hedgehogs, and 50% in jackals (6). In six coastal cities in Algeria, prevalence rates of CanL ranged from 11% to 38%, increasing from west to east. Bejaia had the greatest rate, strongly correlated with the incidence of visceral leishmaniasis (VL) in humans. Because of the absence of a centralized national database and regular screening programs in Algeria, the available epidemiological information regarding CanL is severely limited. Faced with this situation, researchers (in this country) have constantly been led to conduct surveys with the aim of improving the epidemiological mapping of CanL in Algeria (7).

The first goal of this study was to determine the seroprevalence of asymptomatic *Leishmania* infection in a group of clinically healthy owned dogs (dogs that do not exhibit characteristic signs of leishmaniasis) in the wilaya (province) of Constantine, northeast of Algeria, using the indirect ELISA (Enzyme-Linked Immunosorbent Assay) method (ID Screen® Leishmaniasis Indirect, IDvet France) (8, 9). The second objective was to conduct a retrospective epidemiological survey on VL and cutaneous leishmaniasis (CL) in humans in the Constantine region from 2016 to 2024, collecting epidemiological data from the Constantine Public Health Department.

Materials and Methods

Study area

The study was carried out in the wilaya of Constantine, in north-eastern Algeria, at a latitude of 36°14" N to 36°50" N and a longitude of 6°40" E to 6°80" E, and it's bordered by these wilayas: Skikda to the north, Guelma to the east, Oum El Bouaghi to the south, Mila to the west, and Jijel to the northwest (Fig. 1). The province of Constantine covers an area of approximately 2,297.2 km² and is situated at an average altitude of 600–800 m (9). Constantine has a semi-arid, cold climate with hot, dry summers and cold, rainy, wet winters. Annual rainfall ranges from 400 to 600 mm. Temperatures vary from 5.4 °C in the winter to 40 °C in the summer (July), with an average humidity of 25% (4, 10). The wilaya is administratively divided into twelve municipalities (communes): Ouled Rahmoune, Aïn Abid, El Khroub, Ibn Badis, Aïn Smara, Constantine, Ibn Ziad, Hamma Bouziane, Didouche Mourad, Messaoud Boudjriou, Beni Hamiden, and Zighoud Youcef (Fig. 1).

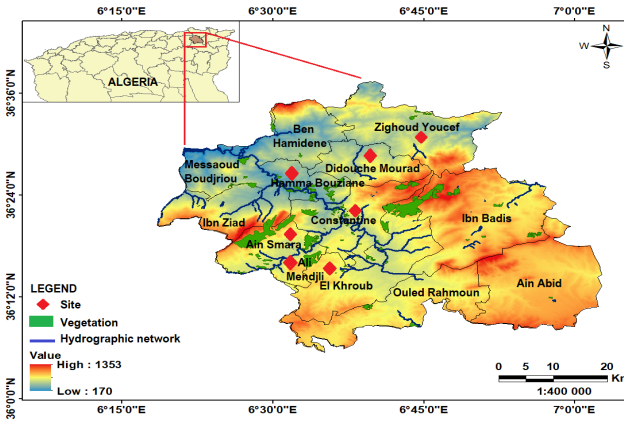

Fig. 1: Geographic map of the wilaya of Constantine (Algeria). The map illustrates some geomorphological information regarding the area as well as the places of origin of the dogs, from which blood samples were collected during the study (shown with red diamonds: El-Khroub, Ali Mendjeli, Zighoud Youcef, Aïn Smara, Hamma Bouziane, Didouche Mourad, and Constantine; personal elaboration, created using ArcGIS Desktop software10.8.2)

Canine leishmaniasis

Sampling

The study, conducted at the Institute of Veterinary Sciences of the University of Constantine, Algeria, was carried out on a group of dogs composed of 84 individuals selected after having been presented for consultation at the carnivore medicine department of the veterinary institute. Between Dec 2022 and May 2023, whole blood samples were collected from the cephalic veins of all dogs selected for the study using red top tubes (no additives; 2-3 ml/dog/tube) after clinical examination and collection of data related to their history (age, sex, breed, and region of origin). To obtain the sera, the tubes containing the blood collected from the dogs were centrifuged directly for 20 min at a speed of 3000 rpm. The sera obtained were then placed in identified Eppendorf tubes (by labelling) and stored at a freezing temperature (-20 °C) until they were used for the serological test.

The animals selected in the study were all over 6 months old and came from the following locations in the Constantine province: El-Khroub (40 dogs), Ali Mendjeli (18 dogs), Zighoud Youcef (10 dogs), Aïn Smara (8 dogs), Hamma Bouziane (4 dogs), Didouche Mourad (3 dogs), and Constantine (1 dog) (Fig. 1).

The selected animals belonged to many breeds (German Shepherds, Malinois, Rottweilers, Dobermans, and various hunting dogs, etc.); however, the majority of the dogs were crossbreeds. Therefore, the analysis of the data in this survey did not take into account the breed of the dogs.

Serological test

The indirect ELISA (ID Screen® Leishmaniasis Indirect, France) was used to detect (or quantify) anti-*L. infantum* antibodies in the 84 sera of dogs selected for the study. According to the manufacturer's instructions, all sera collected, as well as control sera (positive and negative) diluted 1/10 in coating buffer, were deposited in the wells of the ELISA microplate (wells containing a purified L. infantum antigen). After 45 min of incubation at 37 °C, the microplate was rinsed three times with wash buffer. It was then completely covered with the conjugate (anti-dog IgG-peroxidase (horseradish peroxidase) conjugate) diluted 1/10 in conjugate buffer and incubated at 37 °C for 30 min. Three washes were performed immediately following incubation, and a solution containing the chromogenic substrate (TMB) was added to all wells containing the test sera and control sera to detect the conjugate's colorimetric peroxidase reaction. After incubation (in the dark) for 15 min at 21 °C, the peroxidase reaction was stopped by adding the stop solution to the microplate.

The optical density (OD) of each well was read at 450 nm using a microplate reader (Multiskan™ FC Microplate Photometer). For each sample, an S/P% ratio (OD of the sample/OD of the positive control) expressed as a percentage was calculated according to the following formula:

$$\frac{S}{P}\% = \frac{OD_{sample} - OD_{negative\ control}}{OD_{positive\ control} - OD_{negative\ control}} \times 100$$

The S/P% ratio results were interpreted as follows: Positive result: S/P%≥50%; doubtful result: 40%<S/P %<50%; negative result: S/P%≤40.

Human leishmaniasis

To conduct a retrospective epidemiological survey on human leishmaniasis (both cutaneous and visceral) in the Constantine region, epidemiological data relating to this disease, covering the period from 2016 to 2024, were collected from the Public Health Department of the wilaya of Constantine. The data collected were used to calculate the overall incidences of CL and VL in Constantine, as well as the incidences per year and per sex, which were then analyzed and discussed.

Confirmation of the human leishmaniasis cases recorded in the retrospective survey was performed at the University Hospital of Constantine. Given the endemic character of leishmaniasis in Algeria, most laboratories confirmed the disease by microscopy, detecting amastigotes on skin samples (for CL) and bone marrow samples (for VL) colored with Giemsa. However, specialized laboratories, particularly at the University Hospital of Constantine, utilized PCR and indirect serology techniques (such as ELISA) to diagnose VL (11).

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics ver. 20.0 (IBM Corp., Armonk, NY, USA). A chi-square test of independence was conducted to assess the presence of possible significant differences in the rates of *Leishmania* infection seropositivity in dogs (comparison between different municipalities in Constantine, different age groups of animals, and between the two sexes) as well as in cases of human cutaneous and visceral leishmaniasis (comparison between CL and VL over the period 2016–2024 and difference in incidence between sexes for CL and VL). The significance threshold was set at $P<0.05$.

Results

Canine leishmaniasis

The seroprevalence of asymptomatic *Leishmania* infection in the dogs selected for our investigation was 8.33% (7 positive cases/84 dogs) (Table 1). Only one sample (1/84; 40%< S/P %<50) was considered a doubtful result, while 76 samples (76/84) tested negative. The incidences of infection per municipality were as follows :10% (4/40) in El-Khroub, 11.11% (2/18) in Ali Mendjeli, and 10% (1/10) in Zighoud Youcef. No positive cases were reported in other localities (Constantine: 0/1, Didouche Mourad: 0/3, Hamma Bouziane: 0/4, and Aïn Smara: 0/8). Statistical analysis did not reveal any significant difference ($P>0.05$) in the incidence of canine leishmaniasis infection between the selected municipalities, indicating a relatively homogeneous distribution of positive cases throughout the study area.

The chi-square test revealed a highly significant difference ($P<0.05$) in infection rates between age groups: the proportion of positive cases increased markedly with age, from 0% in young animals (6 months to<1 year) to 5% in those aged 1 to 3 yr, and then to 33% in individuals 3 yr and older.

Numerically, males (5 out of 57, or 8.77%) were more affected than females (2 out of 27, or 7.41%) (Table 2). Although, statistical analysis of the study data showed no significant difference ($P>0.05$) in the number of leishmaniasis-positive cases between sexes.

Table 1: Distribution of dogs seropositive for *Leishmania* by municipality

Municipality	Number of tested dogs	Number of positive cases (%)
Aïn Smara	8	0
Ali Mendjeli	18	2 (11.11)
Constantine	1	0
Didouche Mourad	3	0
El-Khroub	40	40 (10)
Hamma Bouziane	4	0
Zighoud Youcef	10	1(10)
Total	84	7 (8.33)
χ^2		3.14
***P*-value**		0.791

Table 2: Distribution of dogs seropositive for *Leishmania* by age group and sex

Distribution of *Leishmania*-positive dogs by age group		
Age group	**Number of tested dogs**	**Number of positive cases (%)**
6 months to <1 year	29	0
1 to 3 yr	40	2 (5)
≥ 3 yr	15	5 (33.33)
χ^2		15.49
***P*-value**		0.00043
Distribution of *Leishmania*-positive dogs by sex		
Sex	**Number of tested dogs**	**Number of positive cases (%)**
Males	57	5 (8.77)
Females	27	2 (7.41)
χ^2		0.00
***P*-value**		1.00

Human leishmaniasis

At the University Hospital of Constantine, the clinical signs of CL generally noted include painless skin lesions (papules, nodules, and crusted ulcers) that can result in scars. LV frequently appeared manifested in severe forms, with symptoms such as prolonged fever, weight reduction, abdominal swelling (splenomegaly/hepatomegaly), anemia, and weakness (unpublished information).

Table 3 illustrates the information on human leishmaniasis in Constantine.

Table 3: Number of cases and incidence (%) of human leishmaniasis (cutaneous and visceral) between 2016 and 2024 in the Constantine region

Year	Number of cases of cutaneous leishmaniasis (%)	Number of cases of visceral leishmaniasis (%)	Total	χ^2	*P*-value
2016	26 (86.66)	4 (13.34)	30		
2017	24 (92.31)	2 (7.69)	26		
2018	13 (100)	0	13		
2019	11 (78.57)	3 (21.43)	14		
2020	10 (100)	0	10		
2021	7 (87.50)	1 (12.50)	8		
2022	13 (92.86)	1 (7.14)	14		
2023	11 (100)	0	11		
2024	9 (90)	1(10)	10		
Total	124 (91.18)	12 (8.82)	136	92.24	< 0.001
Mean ± SD	13.78 ± 6.65	1.33 ± 1.41			

The overall incidence (over the entire study period) of CL was significantly higher than that of the visceral form ($P<0.05$), with 124 cases of CL (91.18%) compared to 12 cases of VL (8.82%).

Throughout the study period, the number of leishmaniasis patients fluctuated year after year. The year 2021 had the fewest cases (8, including 7 CL and only one VL), while 2016 had the highest (30, including 26 CL and 4 visceral cases). No cases of VL were reported during the years 2018, 2020, and 2023.

The distribution of leishmaniasis between the two sexes varied depending on the form of the disease. Statistical analysis revealed that, men were significantly more affected by CL ($P<0.05$) with 78 cases (representing 62.9%) than women (46 cases, or 37.1%). There was no significant difference between the number of VL cases in the two sexes ($P>0.05$). However, women (7 cases, or 58.33%) were slightly (numerically) more affected than men (5 cases, 41.67%). The highest incidence of VL in women (3 cases) was recorded in 2016, whereas the highest incidence of CL in men (19 cases) was recorded in 2017 (Table 4).

Table 4: Number of cases and incidence (%) of cutaneous and visceral leishmaniasis in men and women in the Constantine region from 2016 to 2024

Cutaneous leishmaniasis					
Year	**Men (%)**	**Women(%)**	**Year**	**χ^2**	***P*- value**
2016	16 (61.54)	10 (38.46)	26		
2017	19 (79.17)	5 (20.83)	24		
2018	6 (46.15)	7 (53.85)	13		
2019	7 (63.64)	4 (36.36)	11		
2020	8 (80)	2 (20)	10		
2021	4 (57.14)	3 (42.86)	7		
2022	10 (76.92)	3 (23.08)	13		
2023	4 (36.36)	7 (63.64)	11		
2024	4 (44.44)	5 (55.56)	9		
Total	78 (62.9)	46 (37.1)	124	8.26	0.0041
Mean ± SD	8.66 ±5.45	5.11±2.52			
Visceral leishmaniasis					
Year	**Men (%)**	**Women (%)**	**Total**	**χ^2**	***P*- value**
2016	1 (25)	3 (75)	4		
2017	1 (50)	1 (50)	2		
2018	0	0	0		
2019	2 (66.67)	1 (33.33)	3		
2020	0	0	0		
2021	0	1 (100)	1		
2022	0	1 (100)	1		
2023	0	0	0		
2024	1 (100)	0	1		
Total	5 (41.67)	7 (58.33)	12	0.33	0.5637
Mean ± SD	0.56± 0.73	0.78±0.97			

Discussion

Canine leishmaniasis

The results of this investigation confirmed the high incidence of CanL in Algeria (6, 11). A meta-analysis of animal leishmaniasis in Algeria, based on 12 studies published between 2004 and 2022 and including 4,812 dogs, showed a CanL prevalence of 21.2% (12). Bellatreche et al., using the same ELISA kit as in our research, observed a 10% seroprevalence of *Leishmania* infection in a survey of 94 domestic dogs in the wilaya of Bouira, located in north-central Algeria, a region known for its high prevalence of human leishmaniasis (13, 8).

Our findings also indicated that older animals have an increased probability of infection, possibly due to prolonged cumulative exposure to parasites via vectors or a progressive decline in the effectiveness of immune mechanisms with age. The numerical predominance of CanL incidence in dogs older than four years, noted in our cohort, has been statistically demonstrated by previous studies conducted in Algeria and other countries (3, 12, 14, 15). Miranda et al. mentioned the role of age as a risk factor influencing the development of CanL: the incidence of clinical disease showed two distinct age-related peaks: one in young dogs aged 2 to 4 yr and the other in older dogs over 7 yr (2, 16). The balanced distribution of cases between males and females, noted in our survey, suggested that gender has no significant impact on leishmaniasis infection positivity in the study cohort. According to studies, males are more affected by CanL than females (3, 17). Miranda et al. observed a significantly higher proportion of clinical canine leishmaniasis in males (61%) than in females (39%) (16).

Human leishmaniasis

Algeria is among the countries most affected by human leishmaniasis, where the disease was developing in an epidemic mode, with over 20,000 cases recorded annually and an incidence of 28.19 per 100,000 inhabitants (18,19). Constantine is recognized as an endemic region for leishmaniasis, particularly CL. Overall, 104 cases of human leishmaniasis (102 cases of CL and 2 cases of VL) were reported from the Constantine region during a five-year period (2006–2010) (18). The results on human infection demonstrated the high incidence of CL in the Constantine region, as well as the presence of the visceral form. The latter, although less frequent, remains a major public health problem. The annual global incidence of CL is higher than that of VL; according to the WHO estimates, there were between 500,000 and 1,000,000 new cases of CL worldwide each year, compared to 50,000 to 90,000 new cases of VL (20, 21); in Iran, for example, Mohebali et al. observed a low incidence of VL (less 3%) between 2013 and 2022 (22). In Algeria, the incidence rates of the two disease forms estimated per 100,000 inhabitants in 2020 at 24.23 for CL and 0.05 for VL (11).

The incidence of human leishmaniasis varied from year to year during the duration of our investigation. Fellahi et al. analyzed data on zoonotic cutaneous leishmaniasis (ZCL) in Algeria from 2008 to 2018 and observed peak incidences in 2009, 2011, and 2017 (23). However, in the province of Sétif (a wilaya adjacent to Constantine), the highest incidences were 9.46 cases per 100,000 inhabitants in 2010, 8.85 cases in 2011, and 9.34 cases in 2018. These variations could be attributed to changes in diagnostic and control methods implemented by the Algerian government over the years (23).

Our results showed no significant difference between the number of cases of VL between the two sexes. However, most prior studies conducted in many countries, including Algeria, revealed that VL is more frequent in men than in women (13, 24-26). Data reported by the WHO between 2014 and 2020, in countries with high rates of the disease,

confirms that VL was more common in men than in women; in 2020, the same source reported that 58% of VL cases were attributed to men and 42% to women (27). Male gender is a risk factor for VL and most likely plays a significant role in the infection's development (24).

Men were statistically the most affected by CL in our survey. The gender-based distribution of CL varies by region, with many studies showing a male predominance (5, 10, 27, 28). However, some studies indicate a similar distribution, or even a female predominance in certain age groups or areas (27). According to worldwide data published by WHO, in 2017 and 2018, men (50% and 52%, respectively) were more impacted by CL than women (45% and 47%, respectively) in the countries most affected by this form of the disease (27). A retrospective study conducted over a period of 16 years (between the years 2006 and 2021) in the region of Djelfa (located in the central part of northern Algeria) showed that CL was frequent in male patients (53.60%) (29). Gender differences in the incidence and severity of infection can be attributed to sex-related genetic, physiological, and social factors (4,30).

Sanitary measures, such as stray dog control and disinsectization campaigns, are required to combat CL and VL in Algeria. Molecular and phylogenetic studies of the various *Leishmania* species and their vectors in endemic regions of Algeria, especially the Constantine region, should be performed in order to better understand the relationship between the parasite, humans, dogs, and sandflies. In order to improve diagnosis, standardize diagnostic techniques, and enhance the care of patients, Zeinali et al. suggested creating a network of laboratories for CL (26).

Conclusion

The investigation demonstrates a significant incidence of CanL in asymptomatic canines, as well as CL and VL in humans in the Constantine region, confirming the endemic character of the infection in Algeria, as previously described. Further molecular studies to identify the *Leishmania* species in dogs, sandflies, and humans in endemic areas of Algeria are needed to establish correlations between human and animal infections, allowing for the development of more coordinated control strategies against this persistent disease in Algeria.

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Conflict of Interest

The authors declare that there is no conflict of interest

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