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Research Paper

Seroepidemiology of Canine Visceral Leishmaniasis in Dezful, Iran

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ABSTRACT

Introduction: *Leishmania infantum*-induced canine visceral leishmaniasis (CVL) is endemic throughout the Mediterranean region and constitutes a neglected zoonotic infection that threatens public health. This investigation, employing the DAT technique, assessed prevalence of VL antibodies in symptomatic and asymptomatic domestic and stray dogs in Dezful County and its surrounding areas.

Materials & Methods: For this purpose, serum samples from 130 dogs in Dezful County (105 from shelters and 25 from a veterinary clinic) were collected to assess anti-*L. infantum* antibodies using the direct agglutination test (DAT).

Results: Overall, 16 dogs (12.3%) were seropositive at ≥1:320 and 114 dogs (87.7%) were seronegative at ≤1:320, with 4(3.07%) showing strong positivity at a titer of 1:1280. All seropositive dogs were asymptomatic, with an average age of 1.5 years. A significant difference in seroprevalence was observed between living conditions, with higher rates in stray and sheltered dogs compared to owned dogs (P=0.041).

Conclusion: Our findings, as well as those of asymptomatic seropositive dogs, underscore the potential for an active transmission cycle of CVL in Dezful. Dogs exhibiting clinical signs of leishmaniasis in this region may act as the primary reservoir hosts of *L. infantum* for humans and other susceptible animals. These findings underscore the critical need for ongoing surveillance, integrated vector management, and molecular studies to confirm circulating *Leishmania* species. This study highlights the urgency of targeted control strategies to reduce zoonotic transmission in northern Khuzestan.

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1. Introduction

Visceral leishmaniasis (VL), one of the most clinically significant forms of leishmaniasis in both humans and canines, represents a primary public health concern in Iran, ranking among the most important zoonotic diseases and predominantly affecting children under five years of age [1]. The disease's etiological agent belongs to the *Leishmania donovani* complex (Order: Kinetoplastida) and is transmitted by several species of female sandflies [2]. In the Mediterranean region, *Leishmania infantum* is recognized as the primary causative agent of VL in both humans and dogs. VL is endemic in multiple regions of Iran, including Fars, Bushehr, Kerman, Ardabil, East Azerbaijan, Qom, and North Khorasan provinces [3]. Sporadic cases have also been reported in other parts of the country [4]. In endemic areas of Iran, domestic dogs (*Canis familiaris*) act as the main reservoirs of zoonotic visceral leishmaniasis (ZVL). The high prevalence of canine VL (CVL), including asymptomatic cases, contributes significantly to human transmission. Due to the heavy cutaneous parasitism in infected dogs, whether symptomatic or not, they serve as effective reservoir hosts for VL [5]. Typical clinical features comprise alopecia, lymphadenopathy, splenomegaly, ocular disorders, onychogryphosis, cachexia, and nasal bleeding. In endemic regions, seroprevalence ranges from 10–37%, whereas molecular investigations have reported infection rates as high as 70% [6, 7]. Detecting *Leishmania* parasites in asymptomatic dogs and wild canids is challenging. Domestic dogs serve as the primary reservoirs of the disease, often remaining asymptomatic for extended periods and, due to their relative resistance to antileishmanial treatments, can facilitate the spread of VL across different regions [8]. Consequently, swift detection and management of reservoir populations—including infected domestic dogs and wild canids—represents one of the most effective strategies for preventing human infection [6]. The direct agglutination test (DAT) offers a cost-effective and straightforward serodiagnostic and seroepidemiological method for VL [9]. Its extensive application in human and animal studies underscores its reliability and practicality, with frequent modifications enhancing its accuracy. *L. infantum* infection is indicated at titers ≥1:800, while VL diagnosis requires titers ≥1:3200 with clinical signs.

Dezful, located in the northern part of Khuzestan Province, possesses a unique epidemiological context—including suitable habitats for sandfly vectors, diverse potential reservoirs, dispersed rural settlements, the absence of effective reservoir control programs, and recurring zoonotic disease transmission—that creates a high-risk environment for CVL. Notably, the Dez and Karkheh rivers provide potential breeding grounds for dogs, representing one of the key factors in stabilizing the local transmission cycle. Studies have also shown that *Phlebotomus alexandri* sandflies, exhibiting semi-domestic and exophilic behaviors, act as probable vectors for VL in Khuzestan and, by infecting dogs, can create a reservoir for human cases [10]. Accurate detection of CVL in both symptomatic and asymptomatic dogs is therefore essential. The DAT provides a reliable and cost-effective method to assess CVL seroprevalence in this distinctive setting, enabling a better understanding of local transmission dynamics and informing targeted control strategies. This study represents a crucial step toward comprehensive knowledge and effective control of VL in the region. Therefore, despite limited resources, we aimed to determine the seroepidemiology of CVL in Dezful for the first time.

2. Materials and Methods

2.1. Study area

This cross-sectional descriptive study was carried out between February and September 2023 in Dezful City, located in the northern part of Khuzestan Province, along with its surrounding rural areas. Serum samples were collected in rinary clinic (n=25), from dogs, either having or lacking clinical signs, resulting in 130 dogs. All dogs were examined clinically by an expert veterinarian to determine any related symptoms, as well as sex and age. The study included a diverse population of dogs, from owned medium-sized to giant breeds, including German Shepherds and native breeds such as Sarabi and Ghaderijani, which were kept as guardians, as well as stray dogs and mixed breeds. All the dogs, whether owned or shelter dogs, were kept outdoors.

2-2. Blood sampling

Canine blood samples (2.5 mL) were meticulously collected from 130 dogs in Dezful City, located in the northern part of Khuzestan Province, and from nearby rural areas. The procedure included cephalic venipuncture, with blood samples gently transferred into 15 mL Falcon tubes and processed within 4 to 10 hours after collection. Samples were centrifuged at 800 g for 5 to 10 minutes, after which the sera were separated and stored at -20˚C. The ages of owned dogs were obtained through interviews with their owners, while the ages of stray dogs were estimated by dental examination, ensuring data accuracy.

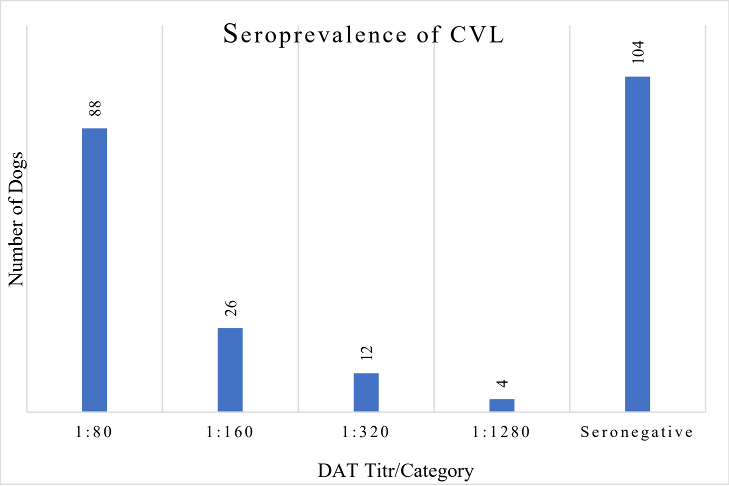

Figure 1. Distribution of DAT titers among 130 examined dogs

Note: The x-axis shows DAT titers/categories, and the y-axis represents the number of dogs. Titers of 1:80 and 1:160 were considered seronegative, while titers ≥1:320 were classified as seropositive for *L. infantum*. Numbers above each bar indicate the absolute frequency of dogs in each category.

2.3. DAT antigen and serological tests

All serum samples were examined using DAT at the respective Protozoology Unit of the School of Public Health, Tehran University of Medical Sciences. The DAT antigen was prepared from *L. infantum* (Lon 49), originally isolated from an infected domestic dog in Iran. Parasites were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, followed by trypsinization, staining with Coomassie brilliant blue, and fixation with 2% formaldehyde. This antigen had previously been validated by World Health Organization (WHO) reference laboratories and shown to be comparable to the standard DAT antigen. According to WHO guidelines and regional studies, an antibody titer of ≥1:320 was considered positive in dogs [9, 11].

2.4. Data analysis

Chi-square and Fisher's exact tests were applied to compare seroprevalence rates by gender, age, and living conditions. Fisher's exact test was used explicitly for small subgroups (e.g. dogs aged 3–6 years). Additionally, 95% confidence intervals (CIs) were calculated to provide more precise seroprevalence estimates. Analyses were performed using SPSS software, version 20, and $P<0.05$ were considered statistically significant.

3. Results

Male dogs comprised 78.3% of the sampled group, while females accounted for 21.7%. All the serum samples were tested using DAT. The overall seroprevalence was 12.3% (16 of 130), and surprisingly, all the positive cases were detected in stray and sheltered dogs without any clinical signs (Figure 1). Anti-*Leishmania*-specific antibodies were considered positive at a cutoff titer of 1:320. Four of 16 seropositive specimens (3.07%) were strongly positive by DAT at a titer of 1:1280, and 12 of 16(9.23%) specimens were considered seropositive at a titer of 1:320.

The prevalence of canine *Leishmania* infection was 13.09% in males and 10.87% in females (Table 1). Statistical analysis, including both chi-square and Fisher's exact tests, revealed no significant difference between male dogs (13.09%, 95% CI, 6.7%, 22.2%) and female dogs (10.87%, 95% CI, 3.6%, 23.6%) ($P=0.712$), confirming the reliability of these findings.

Regarding age groups, the highest seroprevalence was observed in dogs under 3 years old at 12.61% (95% CI, 7.7%, 20.1%), while a prevalence of 10.53% (95% CI, 2.9%, 31.4%) was detected in dogs aged 3–6 years. Although the point estimate in the older group was slightly lower, the wide confidence interval and Fisher's exact test confirmed that the difference between the two age groups was not statistically significant. This finding suggests that younger dogs may be more susceptible to *Leishmania* infection, which could affect vaccination strategies and disease management (Table 2).

Table 1. Seroprevalence of canine *Leishmania* infection by gender

Gender	No. (%)	No.	%
	Tested	DAT Positive	Prevalence (P≥0.05)
Male	84(64.61)	11	13.09
Female	46(35.38)	5	10.86
Total	130(100)	16	12.3

DAT: Direct agglutination test.

Table 2. Seroprevalence of canine *Leishmania* infection by age groups

Age Group (y)	No. (%)	No.	%
	Tested	DAT Positive	Prevalence (P≥0.05)
0–3	111(85.38)	14	12.61
3–6	19(14.62)	2	10.53
Total	130(100)	16	12.3

All seropositive cases were detected among stray and sheltered dogs (15.24%), with a mean age of approximately 1.5 years Analysis by living condition revealed a significantly higher prevalence in stray and sheltered dogs (15.24%, 95% CI, 9%, –23.6%) compared to owned dogs (0%, 95% CI, 0%, 13.7%) (P=0.041). These results underscore the pivotal role of stray and sheltered populations in maintaining the transmission cycle of CVL in the region (Table 3).

Notably, no clinical signs typically associated with *Leishmania* infection were observed in positive cases. Asymptomatic infections hinder early detection. Only one seronegative owned dog showed signs of hair loss around the eyes, and it was diagnosed with dermatophytosis (*Microsporum canis*). After four weeks of topical treatment, the dog responded positively, with no clinical signs observed one month later.

4. Discussion

CVL is a significant zoonotic disease that occurs in both tropical and non-tropical areas and can be fatal to humans and dogs. In areas endemic to ZVL, the rate of *L. infantum* infection in dogs is significantly elevated. The majority of these infected dogs do not display symptoms, with estimates suggesting that more than 50% of seropositive dogs remain asymptomatic. The main focus of our research is the role of asymptomatic dogs in transmitting the infection to humans and other susceptible animals, a crucial aspect that remains unclear. A study by Moshfe et al. (2009) showed that asymptomatic dogs are equally capable as symptomatic dogs in maintaining the *L. infantum* parasite and supporting the domestic transmission cycle in endemic regions [12]. Furthermore, in 1994, Molina et al. highlighted the importance of epidemiological studies on infected animals during the subclinical phase, emphasizing their potential to transmit the parasite despite the absence of clinical symptoms [13]. Earlier studies in Ira-

Table 3. Seroprevalence of canine *Leishmania* infection by living condition: stray and owned dogs

Living Condition	No. (%)	No.	%
	Tested	DAT Positive	Prevalence (P≥0.05)
Stray	105(80.77)	16	15.24
Owned	25(19.23)	0	0
Total	130(100)	16	12.3

nian regions endemic for CVL have found that 13-24% of infected dogs show clinical signs, whereas about 75% remain without symptoms [12]. Mohebali and his colleagues, from 1999 to 2003, showed a positive antibody titer against *Leishmania* in 18.2% of dogs in the western part of the country, including the provinces of Ardabil and East Azerbaijan [14]. Also, in Moshfe's study, 17.4% of dogs had an antibody titer above 1:3200, and 74.4% of seropositive dogs were asymptomatic [12].

This study represents the first epidemiological investigation of visceral *Leishmania* infections carried out in shelters and veterinary clinics in Dezful, located in northern Khuzestan. Contrary to our initial hypothesis that adults, seniors, and symptomatic dogs would be more susceptible to infection and seropositivity, our findings showed differing results: all dogs (12.3%) with antibody titers of 1:320 or higher were asymptomatic. Despite this high proportion within the studied population, our results align with those reported by Moshfe and other researchers [12, 15]. Since most of the population was young (111 out of 130), with an average age of 1.5 years, we cannot definitively conclude that young dogs are more susceptible to being seropositive.

Although international studies have compared shelter versus owned dogs [16, 17], the relevance of regional data is particularly important. In Iran, Mohebali et al. [14] and Moshfe et al. [12] demonstrated comparable trends, with significantly higher seroprevalence among stray and shelter dogs compared to owned animals. Similarly, research conducted in Brazil across 17 shelters reported a seroprevalence of 33.7% (211/627) in shelter dogs, with rates ranging from 25% to 41.2%, whereas earlier studies on domestic dogs reported much lower rates, between 3.4% and 9.6% [16]. Nevertheless, some studies have presented contradictory evidence. For example, Colella et al. observed a higher seroprevalence in domestically owned dogs (31.6%) compared to shelter dogs (14.6%) [18]. Tamponi et al. likewise observed a greater seroprevalence in owned dogs (27.2%) compared to those in shelters (10.6%) [19]. In the current study, the exclusive detection of positive cases among stray and sheltered dogs underscores their potential role as primary reservoirs in Dezful.

The cold, northwestern, and northeastern areas of Iran have been recognized as major hotspots for visceral leishmaniasis, with elevated rates of human *Leishmania* infection observed. Dogs from this zone, with their large populations (7 dogs/100 humans in the Meshkinshahr area) and high infection rates (up to 20% in some villages), are believed to be the primary carriers of the disease, underscoring the need for targeted interventions [14]. The prevalence of *Leishmania* spp. Infection among dogs in the hot southern region was low. Domestic dogs and wild canids are primary reservoirs for *L. infantum* in the Old and New Worlds. Determining the prevalence of canine *Leishmania* infection across various regions of Iran is essential for establishing effective prevention and control strategies for ZVL.

In our study, 16 cases (12.3%) exhibited antibody titers against visceral leishmaniasis exceeding 1:320. No statistically significant association was found between the presence of anti-leishmanial antibodies and the gender of the animals (P=0.712). In the study by Mohabati and colleagues in 2006, the seroprevalence of antibodies against visceral leishmaniasis in 210 blood samples from the dogs studied was reported to be 4.8% [9].

Yahaghi et al. (2022) found that in Khuzestan Province, *Phlebotomus papatasi* and *phlebotomus alexandri* were the dominant sandfly species, accounting for 57.3% and 29.5% of the total population, respectively [10]. The zoonotic risk associated with CVL in Dezful is further heightened by entomological evidence. *Ph. alexandri*, a proven vector of ZVL in Iran, has been reported as the second most abundant sand fly species in Khuzestan Province, including Dezful, Shush, and Shushtar [10, 20]. The coexistence of a 12.3% canine seropositivity rate with a strong vector presence highlights the epidemiological potential for maintaining a zoonotic transmission cycle in the region. This strengthens the public health significance of our findings, particularly for at-risk groups such as children. In this study, we identified 12.3% seropositivity with *L. infantum* among stray dogs without any symptoms of infection by the DAT method. However, Shokri et al. (2017) conducted an updated systematic review and meta-analysis, revealing that in addition to the previously known endemic regions of Iran, CVL is also widespread among the dog population in other provinces, including Khuzestan [21].

5. Conclusion

Our results provide a crucial understanding of the epidemiology of canine leishmaniasis in this region of Iran, which can directly inform the design and implementation of more targeted and effective management and control strategies.

Despite these important observations, several limitations should be acknowledged. The relatively small sample size (n=130) constrained the statistical power for subgroup analyses. Furthermore, the overrepresentation of shelter and stray dogs may have introduced a selection bias, as these animals may not fully represent the broader dog population in Dezful. Consequently, the generalizability of our results should be interpreted with caution. Future studies incorporating larger, more representative samples and molecular confirmation of parasite species are warranted to refine our understanding of the epidemiology of CVL in southern Iran.

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Compliance with ethical guidelines

This study was approved by the Research Ethics Committee of Dezful University of Medical Sciences, Dezful, Iran (Code: IR.DUMS.REC.1397.030).

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Authors' contributions

Conceptualization, study design, project administration, technical, and material support: Ezatollah Ghasemi; Data acquisition: Katayoon Oskouizadeh; Experiments and data interpretation: All authors; Statistical analysis and writing the original draft: Ezatollah Ghasemi and Katayoon Oskouizadeh; Review and editing: Ezatollah Ghasemi and Mehdi Mohebali.

Conflict of interest

The authors declared no conflict of interest.

Data availability

The data that support the findings of this study are available upon request from the corresponding author.

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