



Research Paper

Emerging Evidence of Visceral Leishmaniasis in Kahak
County, Qom Province, Central Part of Iran

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ABSTRACT

Introduction: Visceral leishmaniasis (VL) is a systemic parasitic disease that can be fatal if left untreated. In Iran, *Leishmania infantum* is the causative agent of Mediterranean type of VL, transmitted to humans via the bite of infected *Phlebotomine sandflies* from animal reservoir hosts. This study aimed to introduce a new focus of VL in children up to 13 years old as well as domestic dogs, in Kahak County, Qom Province, during 2022-2023.

Materials & Methods: A descriptive cross-sectional study was carried out involving 316 human serum samples (children under 13 years) and 50 domestic dogs selected through simple random sampling. Blood samples were collected and analyzed using the direct agglutination test (DAT) to detect anti-*Leishmania* antibodies. Samples with anti-*Leishmania* antibody titers of 1:800 or higher were retested after 2-3 weeks for confirmation. Data analysis was conducted using SPSS version 22, utilizing Fisher's exact test, with a significance level of $P < 0.05$.

Results: Out of 316 human samples, five showed suspicious anti-*L. infantum* antibody titers of 1:800, and two samples showed positive titers of 1:1600 and 1:3200, indicating seropositive infection. Among the 50 canine samples, two showed anti-*Leishmania* antibody titers of 1:320. Repeat testing after several weeks confirmed the initial results. The two seropositive human cases were a 7-year-old boy and an 8-year-old girl, both presenting with symptoms such as weakness, paleness, lethargy and hepatosplenomegaly, with no history travelling to other VL endemic areas. These patients were referred to the Health center of the studied area for physical examinations and necessary treatment.

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: **Conclusion:** This study showed that VL occurs at a low prevalence among children up to 13
 : years old in the studied area. Thus, enhancing the awareness of healthcare professionals and
 : public health officials is essential, along with the establishment of a surveillance system for this
 : potentially fatal disease.

1. Introduction

Visceral leishmaniasis (VL), also known as Kala-Azar, is a systemic zoonotic infectious disease caused by intracellular protozoan parasites of the genus *Leishmania* [1]. In Iran, the primary causative agent is *Leishmania infantum*, which is transmitted to humans through the bite of infected female sandflies of the genus *Phlebotomus* [2]. The disease is characterized by severe systemic manifestations and can be fatal if left untreated, with advanced cases often leading to death. According to the World Health Organization (WHO), an estimated 50,000 and 90,000 new cases occur annually worldwide, with over 95% mortality in untreated cases [3].

VL predominantly affects children under ten years of age living in rural areas, with domestic and wild canids, particularly dogs, serving as the main reservoirs in East Mediterranean countries, including Iran [4]. Currently, VL is endemic in several provinces of Iran, including Ardabil, East Azerbaijan, Fars, Bushehr, North Khorasan, and Qom, with sporadic reports from other regions. The first report of an endemic focus of VL in the rural areas of Qom Province was documented in 2001 by Fakhar et al. [5].

Qom Province, located in the central Iran, is bordered by Tehran Province to the north, Semnan Province to the east, Isfahan Province to the south, and Markazi Province to the west. The province is of particular epidemiological importance due to extensive travel and migration for various reasons, increasing the risk of endemic disease transmission [6].

According to records from the Qom Provincial Health Center, several VL cases have been reported among children under five years old in both rural and urban areas over the past decade, confirmed using direct agglutination test (DAT) [7]. Considering the possibility of patients seeking medical care outside the province, there is a critical need to assess the epidemiological status and associated risk factors of VL to support systematic surveillance and disease control within the province. Although VL has previously been studied in some regions of Qom Province, a new focus of VL was identified for the first time in the Kahak district of Qom Province.

2. Material and Methods

2.1. Study area

Kahak is a city located in the central district of Kahak County within Qom Province, Iran, and functions as the capital of both the county and the district. It previously served as the capital of Kahak Rural District until the administrative center was moved to the village of Verjan. According to the 2006 National Census, the population of the city was recorded at 2,766 individuals in 797 households, when it was part of Qom County. The subsequent census in 2011 reported an increase to 2,906 residents living in 883 households (Figure 1).

2.2. Blood sampling

A cross-sectional study was carried out between March 2022 and September 2023, utilizing a randomized cluster sampling technique for sample collection. Written informed consent was obtained from the parents of participating children. A questionnaire was completed by each parent to gather information, followed by the collection of a blood sample from each participant.

Blood samples were obtained from 316 children aged under 13 years in collection tubes. The blood samples were centrifuged at 10000×g for 5 minutes, after which the resulting serum samples were stored at -20 °C. In addition, samples were collected from 50 domestic dogs. All selected dogs underwent a physical examination conducted by a veterinarian. Sampling was carried out under the supervision of a licensed veterinarian and adhered to the ethical standards for animal research. Blood samples were collected from these dogs in the same villages where human samples were obtained. The samples were placed in 10 mL polypropylene tubes and centrifuged at 800×g for 5-10 minutes within 4-10 hours following their collection.

Serum samples were sent to National Reference Laboratory of leishmaniasis, Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Iran, for examination using direct agglutination test (DAT).

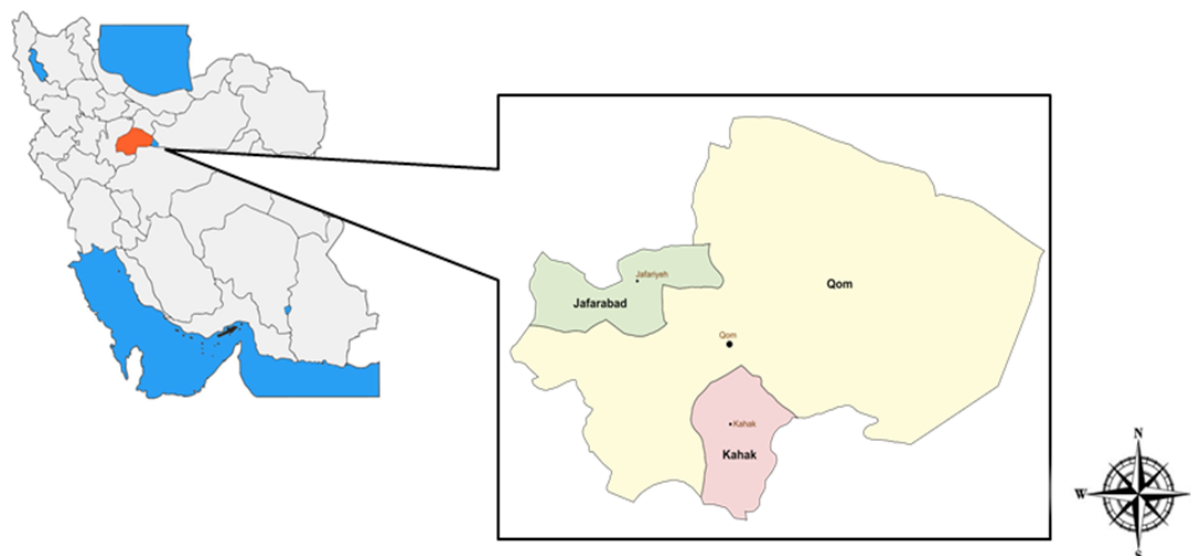


Figure 1. Geographical status of Kahak district in Qom Province

2.3. Serological test

The DAT antigen was made in the Parasitology Unit of the School of Public Health, [Tehran University of Medical Sciences](#). The DAT was conducted following the manufacturer's instructions using 10 μ L of serum. A quantitative DAT was carried out for the titration of anti-*L. infantum*-specific immunoglobulins G (IgG) on sera. Human and dog sera were initially evaluated using DAT. Dilutions of 1:800 and 1:3200 were prepared for human samples, while dilutions of 1:80 and 1:320 were used for dog samples. Samples showing titers of 1:3200 or higher in humans and 1:320 or higher in dogs were further diluted to determine endpoint titers, reaching up to 1:102,400 for human samples and 1:20,480 for dog samples. Each plate was tested daily with negative control wells containing confirmed negative sera and positive control sera. All human samples at 1:800 were considered negative, while those with a titer of 1:3200 or higher were deemed positive. Similarly, dog samples at a dilution of 1:80 were regarded as negative, whereas a titer of 1:320 or above was considered positive [8].

2.4. Statistical analysis

Chi-square and Fisher's exact tests were used to compare seroprevalence values based on gender, and age. Analyses were performed using SPSS- 22 software with confidence interval of 0.95. A $P < 0.05$ was considered statistically significant.

3. Results

Out of 316 human samples, five exhibited suspicious antibody titers of 1:800, and two samples showed positive titers of 1:1600 and 1:3200, indicating seropositive infection. Among the 50 canine samples, two showed antibody titers of 1:320. Repeat testing after several weeks confirmed the initial results. The two seropositive human cases were a 7-year-old boy and an 8-year-old girl, both exhibiting symptoms such as weakness, pallor, and lethargy with hepatosplenomegaly, and confirmed anemia based on CBC tests. These cases were reported to the Qom Provincial Health Center following ethical protocols. Individuals with 1:800 titers showed no apparent clinical or laboratory signs (Table 1).

4. Discussion

The findings of this study provide significant insights into the current status of VL (Kala-Azar) in Kahak County, Qom Province, and an area previously known to have endemic foci of the disease. A zoonotic cutaneous leishmaniasis outbreak caused by *Leishmania major* was identified in the Ghanavat rural district of Qom Province, central Iran, between 1999 and 2001 [9]. The initial reports of Kala-Azar cases in Qom Province, located in central Iran, emerged in 2001, specifically from the villages within the Ghahan district [10].

Table 1. Frequency of positive cases of Kala-Azar according to age and gender in children of villages around Qom in 2022-2023

Characteristics		No.	No. (%)
			Positive Cases
Gender	Male	102	1(0.9)
	Female	214	1(0.4)
Age group (y)	3-6	102	0(0)
	6-12	214	2(0.9)
	Total	316	2(0.6)

In 2014, Rakhshanpour et al. reported that out of 1564 individuals from urban and rural areas, 53 cases (3.38%) showed *Leishmania*- specific antibodies [11]. In 2019, Zanjirani Farahani et al. reported a positive DAT result (greater than 1:3200) leading to the diagnosis of VL in two 2-year-old girls residing in the urban areas of Qom province [10].

Despite efforts to control and monitor VL in Iran, the presence of seropositive cases among children and domestic dogs in this region suggests that the disease remains a public health concern [2]. This discussion will explore the implications of these findings in the context of the study objectives, the broader epidemiological landscape of VL in Iran, and potential strategies for improved disease control.

The study revealed that out of 316 human samples, seven children were seropositive for *L. infantum*, with five showing antibody titers of 1:800 and two showing higher titers of 1:1600 and 1:3200. The presence of seropositive cases in children with no history of travel to other endemic regions strongly suggests that these infections were locally acquired, indicating ongoing transmission within Kahak County. This is particularly concerning given the clinical presentation of these cases, which included symptoms such as prolonged fever, hepatosplenomegaly, anemia, and general weakness. These findings align with the clinical manifestations of VL, underscoring the disease's potential severity and the critical need for timely diagnosis and treatment. The identification of two seropositive domestic dogs with titers of 1:320 further emphasizes the role of canines as a reservoir of *L. infantum* in the region. Given that dogs are a well-known primary reservoir for the parasite, the presence of infected dogs in close proximity to human populations represents a significant risk factor for zoonotic transmission. The finding that these dogs exhibited clinical signs consistent with leishmaniasis, such as alopecia, epistaxis, and wasting, supports the diagnosis and highlights

the need for vigilant monitoring of canine populations in endemic areas.

While our finding confirm the presence of VL in Kahak County, further studies are necessary to assess the true burden and any potential changes in transmission over time. The discovery of new cases in areas outside the historically recognized endemic villages, such as Qahan, indicates that the geographic distribution of the disease may be expanding. This expansion could be attributed to several factors, including increased movement of people and animals between regions, changes in environmental conditions that favor the proliferation of sand-fly vectors, and possibly gaps in the existing disease surveillance and control measures [12].

The seroprevalence rates observed in this study are consistent with findings from other regions in Iran, such as Ardabil, East Azerbaijan, and Fars, where similar studies have reported varying degrees of sero positivity among human and canine populations. For example, several studies have documented the presence of *L. infantum* in both humans and dogs in these areas, with seroprevalence rates ranging from 2% to 10%. The slightly higher seroprevalence observed in this study, particularly among children, may reflect epidemiological differences, including vector transmission intensity and dog population density [13, 14].

Moreover, these findings are in line with global trends in VL, particularly in the Eastern Mediterranean Region, where the disease remains endemic in several countries. The WHO has highlighted the ongoing challenge of controlling VL in these regions, where socio-economic factors, environmental changes, and limited access to healthcare contribute to the persistence of the disease [15]. The similarities between the epidemiology of VL in Kahak County and other endemic areas underscore the need for region-specific strategies that address local challenges.

The continued presence of VL in Kahak County has several important public health implications. First, the identification of seropositive children without severe clinical symptoms at the time of diagnosis suggests that the disease may be underdiagnosed, particularly in its early stages. This underscores the need for increased awareness among healthcare providers, as early detection and treatment are crucial to preventing severe outcomes, including death.

Second, the role of domestic dogs as a reservoir highlights the importance of integrating veterinary public health measures into broader disease control strategy. Regular screening and treatment of dogs, combined with public education campaigns to raise awareness about the risks of canine leishmaniasis, could significantly reduce the reservoir of infection and interrupt the transmission cycle. Additionally, the potential spread disease to new areas within Qom Province and beyond necessitates a coordinated response involving both local and national public health authorities. Surveillance systems need to be strengthened to ensure timely detection of new cases, and vector control measures such as insecticide spraying and insecticide-treated bed nets should be implemented in high-risk areas. The mobility of populations within and across provincial borders also calls for enhanced cross-border collaboration to prevent the spread of VL to non-endemic regions. This study's strengths lie in its comprehensive approach, including both human and animal populations in a region with known endemic foci of VL. The use of the DAT, a reliable serological method with high sensitivity and specificity, ensured accurate detection of seropositive cases [16, 17]. One of the main limitations of the current study is the small number of confirmed cases of VL in both humans and canines out of a larger sample population. Further studies with larger sample sizes and longitudinal follow-up are essential to provide more accurate prevalence estimates and to determine whether this region constitutes a newly established focus of infection. The cross-sectional design provides only a snapshot of disease prevalence at a specific time and does not allow assessment of temporal trends or the identification of risk factors. Additionally, the relatively small geographic area coverage may limit generalizability of the findings to other parts of Qom Province or Iran. Reliance on serological testing also have led to underestimation of subclinical or asymptomatic. Given these findings, several recommendations can be made for future research and disease control efforts. First, longitudinal studies should be conducted to monitor the incidence trends of VL over time and identify potential risk factors for infection.

These studies could provide valuable information on the effectiveness of current control measures and help guide future interventions. Second, there is a need for further research into the genetic diversity of *L. infantum* strains circulating in Iran, as this could have implications for epidemiology, treatment, and vaccine development. Molecular studies could also help clarify the role of different vector species in the transmission of the disease, providing a basis for more targeted vector control strategies. Finally, public health authorities should consider implementing regular screening programs for VL in both human and animal populations in endemic areas, supported by public education campaigns to raise awareness about the disease and promote preventive measures, such as the use of insecticide-treated bed nets and the timely treatment of infected dogs. Enhanced surveillance and reporting systems are also needed to ensure that new cases are detected and managed promptly, preventing further spread of the disease.

5. Conclusion

The present study provides compelling epidemiological evidence of VL transmission in Kahak County, Qom Province, with confirmed cases in both humans and domestic dogs. The co-occurrence of *L. infantum* in canine and human hosts underscores the role of dogs as primary reservoirs in the zoonotic transmission cycle. These findings highlight the emerging risk of VL in central Iran, a region previously underrepresented in national surveillance programs. Given the public health implications, it is critical to implement systematic screening, reservoir control, and vector management strategies. Further longitudinal and molecular studies are warranted to elucidate transmission dynamics and guide targeted interventions. It is essential that the surveillance system be implemented and consistently monitored by physicians and public health managers in the affected areas.

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Compliance with ethical guidelines

The study protocol was reviewed and approved by Research Ethics Committee of [Tehran University of Medical Sciences](#), Tehran, Iran (Code: IR.TUMS.AEC.1402.012). Written informed consent forms were obtained from the parents of participating children, and questionnaires were completed by them.

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Authors' contributions

Conceptualization, study design, and supervision: Mehdi Mohebbali; Data acquisition: Zeinab Ajami; Experiments and data interpretation: Seyed Jafar Adnani Sadati; Statistical analysis: Seyed Mohammad Barani; Writing the original draft: Zeinab Ajami, Zahra Kakooei; Review and editing: Behnaz Akhouni; Project administration, technical, and material support: Bahram Nikmanesh.

Conflict of interest

The authors declared no conflict of interest.

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