Emerging evidence of visceral leishmaniasis in Kahak County, Qom Province, Central part of Iran

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Abstract

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 from Qom Province during 2022-2023.

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 threshold set as at $p<0.05$.

Out of 316 human samples, five exhibited suspicious anti-*Leishmania 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 of them 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 a 8-year-old girl, both exhibiting signs and symptoms such as weakness, paleness, lethargy and hepato splenomegaly 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.

This study showed that VL is observed to have 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 with the establishment a surveillance system for this fatal disease.

Key words: Visceral leishmaniasis, Human, Dog, New focus, Iran

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*, 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, annually, it is estimated that between 50,000 and 90,000 new cases occur worldwide, with over 95% mortality in untreated cases (3).

VL predominantly affects children under ten years of age residing in rural areas, with domestic and wild canids, particularly dogs, serving as the primary reservoirs in East Mediterranean countries, including Iran (4). Currently, VL is endemic in several provinces of Iran such as 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 and colleagues (5).

Qom Province, situated in the central region of Iran, is bordered by Tehran Province to the north, its eastern boundary with Semnan Province, Isfahan Province to the south, and Markazi Province to the west. The province holds significant 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 and confirmed by DAT over the past decade (7). Considering the potential for cases seeking medical care outside the province, there is a critical need to assess the epidemiological status and associated factors of VL for systematic surveillance and disease control within the province. Although VL has been previously studied in some regions of Qom province, but a new focus of VL from the Kahak district from Qom Province was reported for the first time.

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 it functions as the capital of both the county and the district. It served as the capital of Kahak rural district until the capital was moved to the village of Verjan. During the 2006 National Census, the population of the city was recorded at 2,766 individuals residing in 797 households, while it was part of Qom County. The subsequent census in 2011 reported an increase to 2,906 residents living in 883 households (Figure 1).

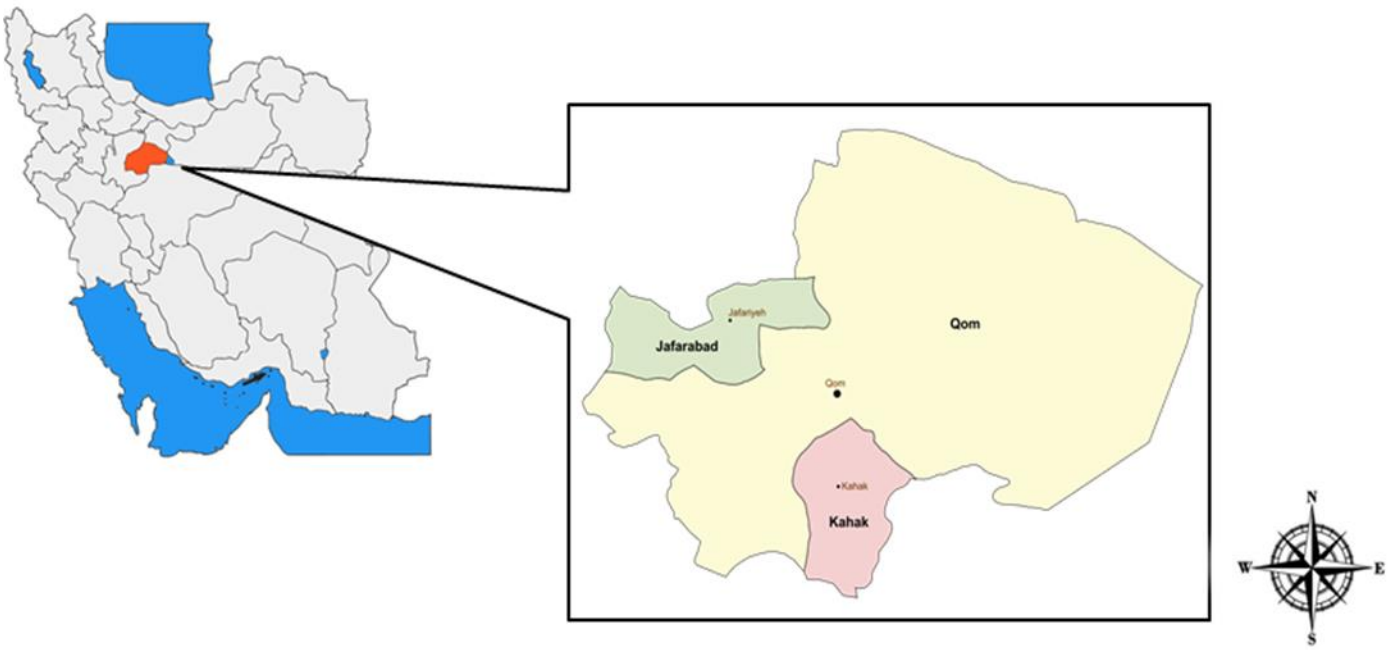

Figure 1. Geographical status of Kahak district in Qom province

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. An informed consent document was obtained from parents of the 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 obtained were centrifuged at 10000 ×g for 5 min after which the resulting serum samples were preserved at −20°C.

In addition, samples were taken from 50 domestic dogs. All the selected dogs underwent a physical examination conducted by a veterinarian. Sampling of dogs 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.

Sera were sent to national reference Laboratory of leishmaniasis, Dept. of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Iran for examination with Direct Agglutination Test (DAT).

2.3. Serological test

DAT antigen was made in the Parasitology Unit of the School of Public Health, Tehran University of Medical Sciences. DAT was conducted following the manufacturer's instructions using 10 μL of serum. A quantitative DAT was carried out for the titration of anti-*L. infantum*-specific immunoglobulins G (IgG) on sera. The human and dog sera were initially evaluated using the DAT for screening purposes; dilutions were prepared at 1:800 and 1:3200 for human's samples also 1:80 and 1:320 for dogs samples. Samples exhibiting titers of 1:3200 in human and 1:320 in dogs were subsequently diluted to achieve end-point titers of 1:102400 for human

samples and 1:20480 for dog samples. Each plate was tested daily with negative control wells containing confirmed negative sera, as well as positive control sera. All human samples at 1:800 were considered negative and 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 exact tests were used to compare sero prevalence values based on gender, age. Analyses were performed using SPSS- 22 software with confidence interval of 0.95. *p* value< 0.05 was considered significant level.

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 hepato splenomegaly, and confirmed anemia through 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).

Table 1: Frequency of positive cases of kala-azar according to age and gender in children of villages around Qom in 1401-1402

Characteristics	Number	Number of positive cases	Percentage of positive cases
Gender			
Male	102	1	0.9
Female	214	1	0.4
Age group			
3-6	102	0	0
6-12	214	2	0.9
Total	316	2	0.6

4. Discussion

The findings of this study provide significant insights into the current status of visceral leishmaniasis (Kala-Azar) in Kahak County, Qom Province, 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). In 2014, Rakhshanpour et al., reported that 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 visceral leishmaniasis in two 2-year-old girls residing in the urban areas of Qom province (10).

Despite efforts to control and monitor visceral leishmaniasis 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's objectives, the broader epidemiological landscape of visceral leishmaniasis in Iran, and potential strategies for improved disease control.

The study revealed that out of 316 human samples, seven children were seropositive for *Leishmania 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 who had not traveled 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, hepato splenomegaly, anemia, and general weakness. These findings align with the clinical manifestations typically observed in visceral leishmaniasis, 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 for *Leishmania 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 visceral leishmaniasis 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 disease's geographic distribution 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 sero prevalence 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 *Leishmania infantum* in both humans and dogs in these areas, with sero prevalence rates ranging from 2% to 10%. The slightly higher sero prevalence observed in this study, particularly among children, may reflect differences in the local epidemiology of the disease, including the intensity of vector transmission and the density of the dog population (13, 14).

Moreover, the study's findings are in line with global trends in visceral leishmaniasis, particularly in the Eastern Mediterranean Region, where the disease remains endemic in several countries. The World Health Organization (WHO) has highlighted the ongoing challenge of controlling visceral leishmaniasis 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 visceral leishmaniasis in Kahak County and other endemic areas underscore the need for region-specific strategies that address the unique challenges of each locality.

The continued presence of visceral leishmaniasis in Kahak County has several important public health implications. Firstly, the identification of seropositive cases in children who did not exhibit 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 in the region, as early detection and treatment are crucial to preventing severe outcomes, including death.

Secondly, the role of domestic dogs as a reservoir for the disease highlights the importance of integrating veterinary public health measures into the 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 for disease spread 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 the use of 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 visceral leishmaniasis to non-endemic regions. This study’s strengths lie in its comprehensive approach, which included both human and animal populations in a region with known endemic foci of visceral leishmaniasis. The use of the Direct Agglutination Test (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 visceral leishmaniasis 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 a snapshot of the disease's prevalence at a specific time but does not allow for the assessment of temporal trends or the identification of risk factors for infection. Additionally, the study was conducted in a relatively small geographic area, which may limit the generalizability of the findings to other parts of Qom Province or Iran. The study's reliance on serological testing alone also means that subclinical or asymptomatic cases

may have been missed, potentially underestimating the true prevalence of the disease given the findings of this study, several recommendations can be made for future research and disease control efforts. First, longitudinal studies should be conducted to monitor the incidence of visceral leishmaniasis 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 *Leishmania infantum* strains circulating in Iran, as this could have implications for the disease's epidemiology and the development of effective treatments and vaccines. 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 visceral leishmaniasis in both human and animal populations in endemic areas. These programs should be accompanied 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.

The present study provides compelling epidemiological evidence of visceral leishmaniasis transmission in Kahak County, Qom Province, with confirmed cases in both humans and domestic dogs. The co-occurrence of *Leishmania 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 is implemented and consistently monitored by physicians and public health managers in the targeted areas.

Acknowledgements

This study was financially supported by Zoonosis Research Center, Tehran University of Medical Sciences, Tehran, Iran (Grant No: 1402-1-213-66165) and Ethic code No.: IR.TUMS.AEC.1402.012.

Authors' Contribution

Study concept and design: M. M

Acquisition of data: Z. A.

Analysis and interpretation of data: SJ. AS.

Drafting of the manuscript: Z. A, Z. K.

Critical revision of the manuscript for important intellectual content: B. A.

Statistical analysis: SM. B.

Administrative, technical, and material support: B. N.

Study supervision: M. M.

Ethics approval

The study protocol was reviewed and approved by Tehran University of Medical Sciences (ethical code: IR.TUMS.AEC.1402.012).

Conflict of interests

We declare that we have no conflict of interest.

Funding/Support

This study was founded and supported by Tehran University of Medical Sciences (TUMS), Tehran, Iran (Project No: 1402-1-213-66165). We thanks from zoonosis Center of Tehran University of Medical Sciences for the confirmation and support. This study is MSc thesis supported by Tehran University of Medical Sciences (TUMS), Tehran, Iran.

Informed Consent

A written informed consent form and a questionnaire were completed by the participant parents.

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