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Review Article

Visceral Leishmaniasis in Iran: An Update on Epidemiological Features from 2013 to 2022

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Abstract

Background: Visceral leishmaniasis (VL) is one of the most important neglected tropical diseases. The zoonotic form of VL is endemic in some areas of Iran. We aimed to determine the status of VL identified in humans and canines in different parts of Iran from 2013 to 2022.

Method: A national representative cross-sectional study was conducted in 10 provinces of Iran, including the national leishmaniasis reference lab. We employed the direct agglutination test (DAT) as a reliable serological method to detect anti-*Leishmania infantum* antibodies in humans and animal reservoir hosts. Additionally, a narrative literature review was conducted to identify relevant studies on VL seroprevalence in Iran from 2013 to 2023.

Results: The results of 21281 human and 5610 canine serum samples from 2013 to 2022 are reported. Altogether, 448 (2.1%, 95%CI: 2.0-2.3) human serum samples showed anti-*L. infantum* antibody levels of ≥1:3200. Of these samples, 13716 (64.5%) were collected actively, which showed a seroprevalence of 0.6% (95% CI: 0.5-0.8) and 7565 (35.5%) were collected passively, which showed a seroprevalence of 4.8% (95%CI: 4.3-5.3). Overall, 1035 (20.1%, 95%CI: 19.0-21.2) of 5160 domestic dogs (*Canis familiaris*) samples showed anti-*L. infantum* antibody levels of ≥1:320. Northwest (۲,۸%) and northeast (0.96%) regions had the highest human VL seroprevalence, while northwest (21.5%) and south (14.4%) regions had the highest canine VL seroprevalence.

Conclusion: Zoonotic VL, an endemic parasitic disease, is still present in several different distinct areas across Iran. While human VL cases have shown a declining trend over the last decade, the prevalence of canine VL remains significant.

Introduction

Leishmaniasis is a group of neglected tropical diseases caused by various species of *Leishmania* parasite. According to the WHO, leishmaniasis is endemic in at least 97 countries, with 12 million infected people and 350 million at risk of infection, and over 20,000 annual mortality (1, 2). The bite of an infected sandfly vector transmits the *Leishmania* infection to both humans and other mammals (3). There are three main clinical manifestations of leishmaniasis: cutaneous, mucocutaneous, and visceral. Visceral leishmaniasis (VL), known as kala-azar is not common form of leishmaniasis, but if left untreated, it is lethal (2). Among tropical diseases, VL ranks second in mortality and seventh in disability-adjusted life years (4, 5).

Compared to cutaneous leishmaniasis, which is endemic in more than half of the provinces of Iran, VL has been observed sporadically in most areas of Iran (6, 7). With almost 100 new symptomatic cases reported annually, VL is endemic in Iran's southern and northwestern regions (8-١٠).

Natural leptomonad infections have been observed in several species of *Phlebotomus* sandflies in different regions of Iran. These include *Phlebotomus* (Lar.) *kandelakii*, *Phlebotomus* (Lar.) *Perfiliewi transcaucasicus*, and *Ph.* (Lar.) *tobbi* in the northwest (١١), as well as *Ph.* (Paraphlebotomus) *caucasicus*, *Ph.* (Lar.) *major*, *Ph.* (Lar.) *alexandri*, and *Ph.* (Lar.) *keshishiani* in the south and central parts of the country.

During the last decade, the direct agglutination test (DAT) has been used extensively for conducting seroepidemiological studies of VL in human and animal reservoirs in various parts of Iran, particularly in the endemic regions (8-10, 12,13). DAT and ELISA have the highest specificity and sensitivity for identifying VL, whereas DAT is a simple, cost-effective, and field-applicable test. Considering the high sensitivity (96%) and specificity (95%) of DAT, it could be recommended for seroepidemiological research as well as early and accurate diagnosis of VL, especially in endemic regions (14-16). The latest comprehensive report on VL in Iran was conducted for the period of 2002-2012, which reported a seroprevalence of 4.7% for human VL (HVL) and 12.2% for canine VL (CanL) (17). Since then, sporadic studies have been conducted, each focusing on a specific district or region of Iran, resulting in limited information on the current distribution of VL in the country.

We aimed to determine the current distribution of VL in different geographical locations of Iran for determining health policy and promotion of VL surveillance. The focus of this study was on infection rates of HVL particularly in children and canines particularly on domestic dogs (*Canis familiaris*) as the most important reservoir hosts of the disease in the Mediterranean region and American continent using the DAT method from 2013 to 2022. The present study provids an updated account of VL in Iran from 2013 to 2022, building upon prior research conducted until 2012 that comprehensively reviewed the disease's epidemiological features up to that point in time.

Methods

Search strategy and keywords

To provide a comprehensive and representative overview of VL seroprevalence in Iran from 2013 to 2022, we conducted a comprehensive literature review with no language barrier to identify relevant studies reporting the seroprevalence of canine or human visceral leishmaniasis in Iran from 2013 to 2023. The search was conducted using electronic databases, including PubMed, Scopus, Web of Science, Google Scholar, Magiran, IranDoc, and Scientific Information Database (SID).

Our search strategy included a combination of keywords and Medical Subject Heading (MeSH) terms related to VL, Iran, and seroprevalence. We also included variations of

these terms and used Boolean operators (AND, OR) to expand the search.

Inclusion criteria

We included studies that reported the seroprevalence of VL in humans (active case detection), canines (*C. familiaris*, *Felis catus*, *C. aureus*, and *Vulpes vulpes*), and sandflies, using the Direct Agglutination Test (DAT) from 2013 up to 2022. Studies that reported seroprevalence through methods other than DAT or in other animals were excluded. For Studies on sandflies, studies were included if they reported data on the method of sandfly collection, the morphology of captured sandflies, and the method of identifying natural *Leishmania* infection (parasitological including, microscopy or/and animal inoculation, and molecular such as comparative DNA sequence of selected gene fragment (18-22)).

Reference Leishmania laboratory data

The "Leishmaniasis Reference Laboratory," Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical School, Tehran, Iran, serves as a pivotal hub for this study. This laboratory plays a vital role in our research by receiving and analyzing specimens from all regions across the country, thereby representing a broad spectrum of the Iranian population. Notably, this center accumulates specimens that encompass a wide array of leishmaniasis cases, which contributes significantly to the comprehensiveness of our analysis.

Alongside the data extracted from the literature review, we have included previously unreported data on DAT results of our laboratory. Our laboratory data and extracted information from external studies provides a more holistic understanding of the epidemiological landscape of visceral leishmaniasis in Iran from 2013 to 2022. For a more detailed exposition of the laboratory methods employed in gathering and processing our own data, please refer to our previously published article (17).

In cases where our laboratory's samples were not available for a specific region and year, we incorporated results from studies identified in the literature search. Additionally, we combined our data for regions where we had collected samples with information extracted from the literature review to provide a more comprehensive picture of seroprevalence in the area.

Results

Human samples

Overall, 21281 human serum samples, 13716 actively and 7565 passively, were reported from 2013 to 2022. Anti-*L. infantum* antibody levels of ≥1:3200 was observed in 448 of 21281 (2.1%, 95%CI: 2.0-2.3) human serum samples (Fig. 1). The seroprevalence for actively and passively collected samples was 0.6% (95% CI: 0.5-0.8) and 4.8% (95%CI: 4.3-5.3), respectively (Table 1). The total VL seroprevalence varied between 1% (2021) and 3% (2018). The seroprevalence of passively collected samples from individuals with suspicious symptoms varied from 1.3% (2022) to 7.6% (2013); moreover, it showed a decreasing trend over the years (Fig. 1). Northwest and northeast regions had the highest seroprevalence, 3.2%, and 1.0%, respectively (Fig. 2). Of 448 DAT+ cases, 362 (80.8%) had symptoms (23-33). Northwest: East Azerbaijan and Ardabil provinces; West: Ilam and Kermanshah provinces; Northeast: Razavi Khorasan and Northern Khorasan provinces; South: Bushehr, Kerman, and Fars provinces; Center: Alborz, Qom, and Semnan provinces; North: Gilan province

Table 1: Seroprevalence of passive and active human visceral leishmaniasis using direct agglutination test (DAT) with anti-*Leishmania infantum* antibodies by geographical zones (2013-2022) in Iran

Year		*Region*	*No. of samples*	*DAT+*	*Seroprevalence (%)*	*Reference*
2013	Passive	Northwest	595	41	6.8	*
		Reference lab	79	10	12.7	*
	Active	West	456	2	0.4	(23)
		Center	1007	2	0.2	(24)
	Total		2137	55	2.6	
2014	Passive	Northwest	654	36	5.5	*
		Reference lab	131	14	10.7	*
	Active	West	872	0	0	(25)
		South	2178	11	0.5	(26)
	Total		3835	61	1.6	
2015	Passive	Northwest	716	36	5	*
		Reference lab	140	20	14.3	*
	Active	Northwest	956	3	0.3	(27, 28)
		South	1386	19	1.4	(29)
	Total		3198	78	2.4	
2016	Passive	Northwest	556	24	4.3	*
		Reference lab	132	14	10.6	*
	Active	Northwest	1420	13	0.9	(30)
		Northeast	422	0	0	*
	Total		2530	51	2.0	
2017	Passive	Northwest	759	31	4.1	*
		Reference lab	88	9	10.2	*
	Active	Northwest	662	5	0.7	*
		Center	1062	6	0.6	*
	Total		2571	51	2.0	
2018	Passive	Northwest	970	28	2.9	*
		Reference lab	104	9	8.6	*
	Active	Northwest	231	2	0.9	*
	Total		1305	39	3.0	
2019	Passive	Northwest	795	32	4.0	*
		Reference lab	78	5	6.4	*
	Active	Northeast	306	7	2.3	*
		Center	504	0	0	(31)
	Total		1683	44	2.6	
2020	Passive	Northwest	535	25	4.7	*
		Reference lab	54	6	11.2	*
	Active	North	918	8	0.9	(32)
	Total		1507	39	2.6	
2021	Passive	Northwest	422	7	1.7	*
		Reference lab	55	6	10.9	*
	Active	Northwest	250	0	0	*
		West	900	3	0.3	(33)
	Total		1627	16	1	
2022	Passive	Northwest	637	4	0.6	*
		Reference lab	65	5	7.7	*
	Active	South	186	5	2.7	*
	Total		888	14	1.6	

Seroprevalence data is presented as % (95% confidence interval); DAT: Direct agglutination test.
* Original data of this study Proven or Probable vectors of VL in Iran

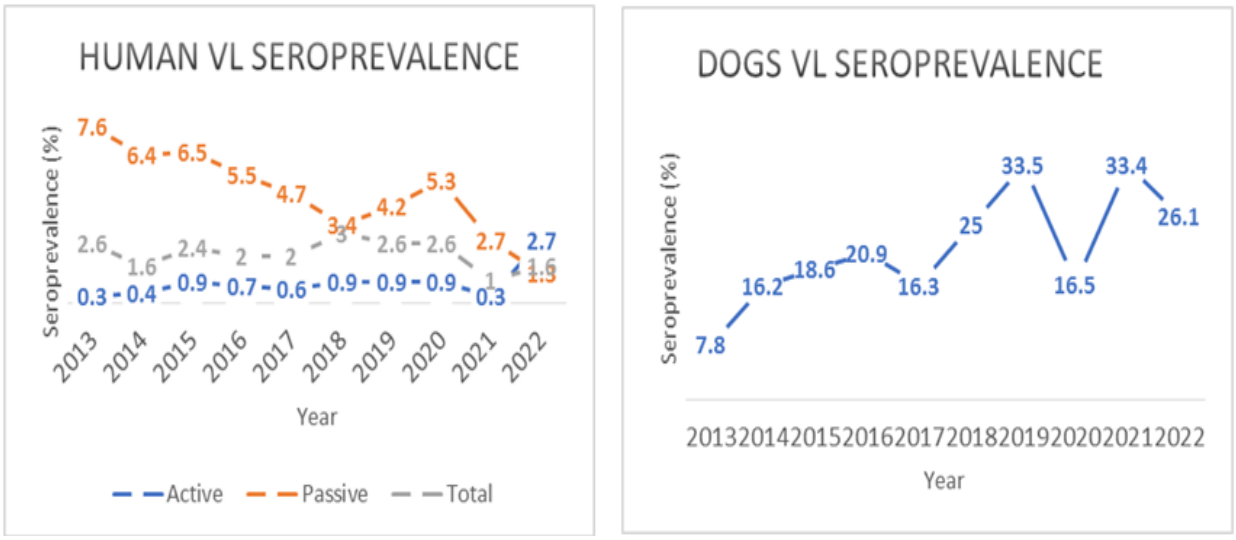

Fig. 1: Human and canine VL seroprevalence trend in Iran from 2013 to 2022 (data from our laboratory and references 23-41)

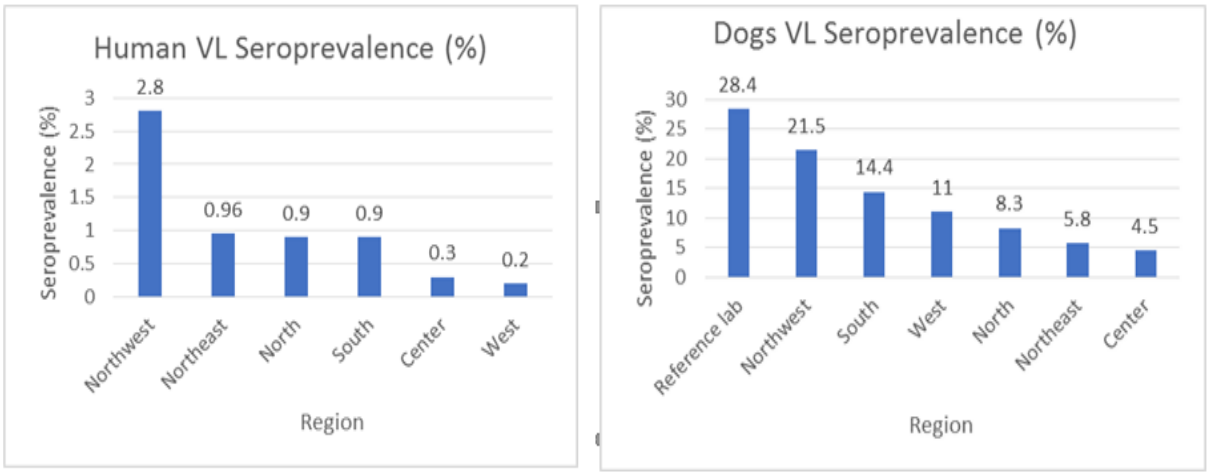

Fig. 2: Human and canine VL seroprevalence in different regions of Iran from 2013 to 2022 (data from our laboratory and references 23-41)

Canine samples

Overall, 5610 reservoir host serum samples (5160 *C. familiaris*, 195 *F. catus*, 180 rodents, 69 *C. aureus*, and 6 *Vulpes vulpes*) were reported from 2013 to 2022. Overall, 1035 (20.1%, 95%CI: 19.0-21.2) of the dog samples showed anti-*L. infantum* antibody levels of ≥1:320 (Table 2). The VL seroprevalence for dogs ranged from 7.8% (2013) to 33.5% (2019), Fig. 1. Northwest and South regions had the highest CanL seroprevalence, 21.5%, and 14.4%, respectively (Fig. 2) (25, 29, 39, 34-38). Northwest: East Azerbaijan and Ardabil provinces; Center: Chaharmahal Bakhtiyari and Semnan provinces; South: Fars and Kerman provinces; West: Ilam and Lorestan provinces; Northeast: Northern Khorasan province; North: Gilan province.

Table 2: Dogs *(Canis familiaris)* visceral leishmaniasis using direct agglutination test (DAT) with anti-*Leishmania infantum* antibodies by geographical zones (2013-2022) in Iran

Year	*Region*	*No. of samples*	*DAT+*	*Seroprevalence*	*Reference*
2013	Center	535	32	6	*
	Northeast	3	2	66.7	*
	South	80	3	3.7	(34)
	Reference lab	23	13	56.5	*
	Total	641	50	7.8	
2014	West	52	4	7.7	*
	Reference lab	47	12	25.5	*
	Total	99	16	16.2	
2015	West	52	11	21.1	(25)
	Northwest	5	5	100	*
	South	165	13	7.9	(29)
	Reference lab	100	31	31	*
	Total	322	60	18.6	
2016	Northwest	68	17	25	(35)
	Reference lab	277	55	19.8	*
	Total	345	72	20.9	
2017	Northwest	451	107	23.7	(30, 36)
	Center	443	12	2.7	(37)
	Reference lab	243	66	27.2	*
	Total	1137	185	16.3	
2018	Northwest	11	6	54.5	*
	West	96	7	7.3	*
	South	60	28	46.7	*
	Northeast	200	8	4.0	*
	Reference lab	266	109	41.0	*
	Total	633	158	25.0	
2019	Northwest	14	8	57.1	*
	Reference lab	246	79	32.1	*
	Total	260	87	33.5	
2020	Northwest	283	16	5.6	(38)
	Reference lab	433	102	23.5	*
	Total	716	118	16.5	
2021	Northwest	45	30	66.7	*
	Reference lab	308	88	28.6	*
	Total	353	118	33.4	
2022	Northeast	4	2	50	*
	North	60	5	8.3	*
	Reference lab	590	164	27.8	*
	Total	654	171	26.1	

Seroprevalence data is presented as % (95% confidence interval); DAT: Direct agglutination test.
* Original data of this study

From the years 2013 to 2022, the number of samples from canines other than dogs was relatively limited. In 2015, two studies on cats (*F. catus*) in the northwest region reported a sample size of 195, with 20 cases (10.3%) testing positive for DAT (39, 40).

A study conducted in 2013 on jackals (*Canis aureus*) in the northeast region included a sample size of 60, with 7 cases (11.7%) testing positive for DAT (41). In 2018, our gathered data on jackals in the northwest region indicated a sample size of 9, with 2 cases (22.2%) testing positive for DAT. A study conducted in 2013 on foxes (*V. vulpes*) in the northeast region included a sample size of 6, with 4 cases (66.7%) testing positive for DAT (41). Our gathered data from 2014 on rodents in the northwest region showed a sample size of 180, with 2 cases (1.1%) testing positive for DAT.

Vectors

During the last three decade, epidemiological, molecular, and parasitological studies in VL endemic regions of Iran showed that *Ph. perfiliewi transcucasicus*, *Ph. kandelakii*, and *Ph. tobbi* in northwestern Iran, *Ph. keshishiani, Ph. major s.l., and Ph. alexandri* in southern regions, *Ph. kandelakii* in northeastern, and *Ph. tobbi* in northern regions of Iran are acknowledged as probable or confirmed VL vectors. Infection rates were 0.3 to 3.4 for *Ph. kandelakii*, 0.9 to 2.8 for *Ph. perfiliewi transcucasicus,* 1.2 to 25 for *Ph. tobbi*, 3 to 8.3 for *Ph. major s.l.,* 1.7 to 4.2 for *Ph. alexandri*, and 1.1 for *Ph. keshishiani* (18-22, 42-52). Table 3 shows *Leishmania* infections in *Phlebotomus* spp. in VL-endemic parts of Iran from 1992 to 2022.

Table 3: Proven or Probable vectors of VL in Iran by geographical zones (1992-2022)*

Zone	*Province*	*District*	*Phlebotomus Spp.*	*Infection rate (%)*	*Leishmania species*	*Isolation method*	*Reference*
	Ardabil	Meshkin-Shahr	*Ph.(Lar.) kandelakii*	0.3	*L.infantum*	Parasitology	Nadim et al. 1992 (42)
	Ardabil	Meshkin-shahr	*Ph.(Lar.) kandelakii*	1.1	*L.infantum*	Nested-PCR	Rassi et al. 2005(43)
	North Khorassan	Shirvan	*Ph.(Lar.) kandelakii*	3.4	*L.infantum*	PCR-RFLP	Rassi et al. 2012(44)
	Ardabil	Germi	*Ph.(Lar.) perfiliewi transcaucasicus*	0.9	*L.infantum*	Parasitology	Nadim et al. 1992(42)
	Ardabil	Germi	*Ph.(Lar.) perfiliewi transcaucasicus*	1.1	*L.infantum*	PCR	Rassi et al. 2009(18)
Northwest	Ardabil	Bilesavar	*Ph.(Lar.) perfiliewi transcaucasicus*	1.5	*L.infantum*	PCR-RFLP	Sanei Dehkordi et al. 2011(45)
	Ardabil	Germi	*Ph.(Lar.) perfiliewi transcaucasicus*	0.94	*L.infantum/ L. donovani*	Semi-nested PCR	Oshaghi et al. 2009(46)
	East Azerbaijan	Kalibar	*Ph.(Lar.) perfiliewi transcaucasicus*	2.85	*L.infantum*	Nested PCR	Parvizi et al. 2008(47)
	East Azerbaijan	Azar-Shahr	*Ph.(Lar.) tobbi*	25(1)	*L.infantum*	PCR-RFLP	Oshaghi et al.

							2013(48)
	Ardabil	Bilesavar	*Ph.(Lar.) tobbi*	6.25	*L.infantum*	PCR-RFLP	Rassi et al. 2012(49)
	Ardabil	Bilesavar	*Ph.(Lar.) per-filiewi trans-caucasicus*	1.47	*L.infantum*	PCR-RFLP	Rassi et al. 2012(49)
North	Alborz	Savodjbolagh	*Ph.(Lar.) tobbi*	1.25	*L.infantum*	PCR-RFLP	Bahrami et al.2014(50)
	Fars	Ghir-Karzin	*Ph.(Lar.) keshishiani*	1.1	*L.infantum*	Parasitology(2)	Seyedi-Rashti et al. 1995(22)
	Fars	Ghir-Karzin	*Ph.(Lar.) major S.l.*	3-5	*L.infantum*	Parasitology(2)	Sahabi et al. 1992(21)
	Fars	Ghir-Karzin	*Ph.(Lar.) major S.l.*	8.3	*L.infantum*	Nested PCR	Azizi et al. 2008(19)
South	Khuzestan		*Ph.(Lar.) alexandri*	1.7	*Leishmania spp.*	Parasitology(3)	Javadian et al. 1997(51)
	Fars	Nourabad Mamasani	*Ph.(Lar.) alexandri*	4.2	*L.infantum*	Parasitology(3) Semi-nested PCR	Azizi et al. 2006(20)
	Fars	Farashband	Ph. (Lar.) papatasi	0.76	L.infantum/ L.major	Nested PCR	Rassi et al. 2013(52)

* Table 3 presents an update to the previous table published by Mohebali (17), providing additional information on

1- Of eight female Ph. tobbi, 2 (25%) were found naturally infected with L. infantum.

2-Leishmania sp. was inoculated into golden hamsters intraperitoneally and produced VL infection that confirmed by microscopy

3-Natural promastigote infection was found

Discussion

In this study, we reviewed the VL seroprevalence rate in different geographical locations of Iran between 2013 and 2022. The human VL (HVL) seroprevalence was 2.1% overall, 4.8% for passively, and 0.6% for actively collected samples. Seroprevalence among canines was approximately 20.1%. Northwest and northeast had the highest HVL seroprevalence, while the northwest and south had the highest CanL seroprevalence. Despite fluctuations, a declining trend in HVL was observed during the study period.

Comparable research, examining HVL cases from 2002 to 2012 (a period of 11 years) in Iran, reported a seroprevalence rate of 4.7%, which is higher than our findings (Fig. 3) (17). Nonetheless, in a meta-analysis of relevant articles published between 1995 and 2019, an HVL seroprevalence of 2% was revealed (95% CI: 1-2%), which is consistent with our findings (53). Similar to us, Rostamian et al presented a declining trend in HVL seroprevalence over time, which may account for the higher seroprevalence observed in the aforementioned study (17). Moreover, the WHO report on leishmaniasis revealed a decrease in VL cases globally and in all regions (54). This decrease in seroprevalence could be mainly attributable to improvements in hygiene and living circumstances (55, 56).

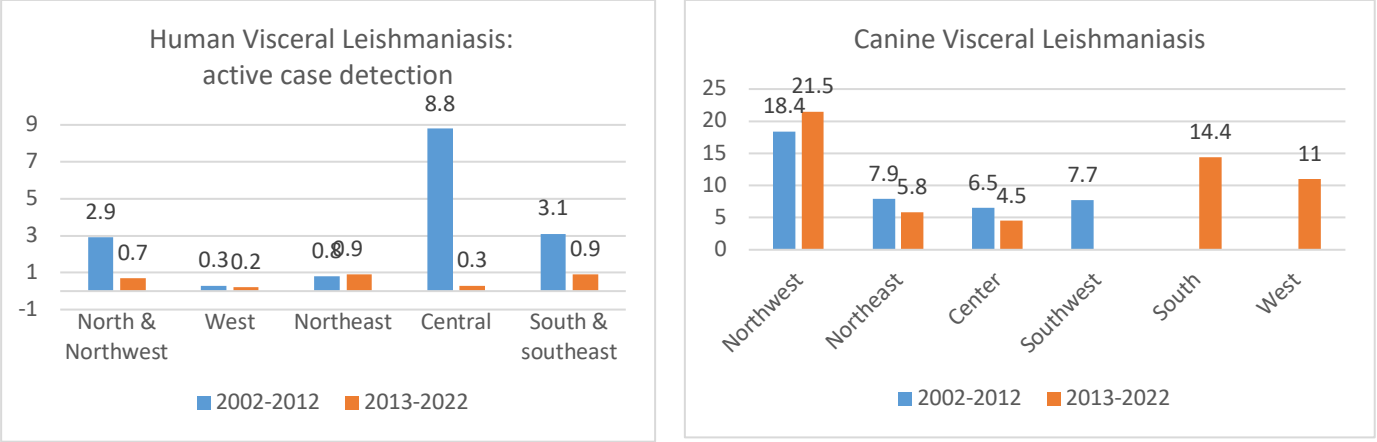

Fig. 3: A decadal comparison of visceral leishmaniasis seroprevalence in Iran
This bar graph presents a decadal comparison of visceral leishmaniasis seroprevalence in Iran from two time periods: 2002-2012 (data from Mohebali (17)) and 2013-2022 (data from our laboratory and references 23-41)

Following data from 2000 to 2012 (17), the northwestern regions of Iran still had the highest HVL seroprevalence in the recent decade (2012-2022), Fig. 3. Even older studies (1985-1999) revealed that the northwest had the highest HVL incidence rate (57).

The seroprevalence of CanL was 20.1%, while data from 2002-2012 indicated a seroprevalence of 12.2%, Fig. 3 (17), and a meta-analysis of studies from 1982 to 2015 found a pooled seroprevalence of 16% in dogs and 10% in jackals, wolves, and foxes (58). A systematic review in Iran also reported a prevalence rate of 4% to 32%, with a higher prevalence in endemic areas (59). The higher prevalence in this study could be due to different sampling methods for canines. We evaluated CanL by analyzing samples of symptomatic canines, whereas, the previous study in Iran (17) used a non-probable occasionally multistage cluster method to sample the canines. However, in agreement with our findings, northwest areas of Iran showed the greatest CanL seroprevalence.

The declining trend observed for HVL seroprevalence was not observed for CanL. The difference between the declining seroprevalence of HVL and the increasing rate of CanL may be attributed to several factors. First, the different clinical presentations of the disease in humans and dogs may contribute to this disparity, as changes in HVL seroprevalence may not reflect disease incidence in dogs. Second, differences in surveillance systems for human and canine VL, including methodologies and reporting mechanisms, affect the comparability of data. Additionally, variations in exposure and risk factors between humans and dogs play a role, with changes in human behavior or environmental factors potentially reducing the risk in humans while increasing in dogs. Furthermore, differences in the effectiveness and implementation of intervention strategies targeted at human VL may result in an increase in canine VL rates despite declining human seroprevalence. Lastly, a time lag between changes in human and canine VL rates can influence these trends, with longer-term changes taking more time to manifest in canines.

In the previous review study of Iran, the seroprevalence of CanL was much higher than the HVL seroprevalence (17). Considering the declining trend in HVL in our study similar to global data, and not observing the same trend for CanL in our study, the higher CanL preva-

lence than HVL can be explained. Moreover, while hygiene and living circumstances, factors contributing to HVL seroprevalence, are getting better (55, 56), the distribution of vectors including sandfly vectors is expanding due to global climate change (60-62), this can explain the higher CanL prevalence and also the declining trend in HVL seroprevalence and not CanL. In addition, it was often believed that vectors, animal reservoirs, and human hosts are vulnerable to infection by a single type of *Leishmania* parasite, however, new studies using next-generation sequencing found that novel vector-parasite-reservoir associations increase the permissiveness of vectors and reservoirs to specific *Leishmania* species, hence enhancing the occurrence of coinfections (63). This may explain the substantially greater prevalence of CanL compared to HVL and necessitates more NGS-based studies on CanL and HVL genotypes.

There is an association between HVL incidence and CanL prevalence (64). In VL endemic regions, dogs are the potential reservoirs of the disease (65, 66). All these demonstrated the importance of monitoring and controlling the CanL seroprevalence in managing and eradicating HVL.

Other important factors influence VL management. Co-morbidities are one of the major contributing factors (62, 67). Several studies have conclusively proven immunosuppression and malnutrition as risk factors for VL infection and the most significant is HIV (62, 68). VL-HIV co-infection was initially identified in Spain (69) and had been reported in Iran (70, 71).

More than 70% of our DAT+ cases had at least three clinical symptoms of pallor, fever, and abdominal distension. The 2000-2012 data also showed a similar frequency of 75% for symptomatic DAT+ cases (17). This demonstrates that approximately 30% of DAT+ cases are asymptomatic, indicating the need for additional molecular tests for diagnosing VL in these individuals. Moreover, considering that asymptomatic cases do not require treatment, the asymptomatic rate is important to estimate the expenses of providing therapeutic and diagnostic procedures.

Comparing VL status in recent decades to the previous decade is important. HVL has shown a decrease in all regions in Iran in the recent decade compared to the previous decade, Fig. 3. This decline may be attributable to a number of factors. First, urbanization and rural depopulation may have played a role. Considering that VL is primarily a rural and nomadic disease, the decline in population and the shift in occupation of some nomads influenced by decrease in rainfall and climate change in the last decade likely contributed to this decline.

Improvement in living conditions and infrastructure facilities in many villages and cities such as the provision of gas may have reduced disease-carrying vectors, thereby decreasing the prevalence of VL.

In addition, increased awareness and improved health knowledge among the population, as well as timely referral to urban and rural health centers, likely contributed to the containment of VL. This enhanced knowledge of the disease, its symptoms, and preventive measures could have led to early diagnosis and treatment, thereby contributing to the decline in VL cases.

Moreover, in collaboration with the Infectious Disease Management Center of Iran's Ministry of Health and the Leishmaniasis Reference Laboratory, the development of the DAT method for the diagnosis of kala-azar in endemic regions has been a decisive factor. This simple and dependable diagnostic method enables precise and expeditious identification of infected individuals, thereby facilitating targeted interventions and control.

However, canine visceral leishmaniasis has shown an increase in the northwest and south regions in the recent decade compared to the previous decade, Fig. 3. This upward trend can be attributed to several factors. First, there is a noticeable lack of case identification and providing adequate care for leishmaniasis in

dogs. In Addition, time and effective treatment for leishmaniasis in dogs residing in rural areas is often inadequate, primarily due to its exclusion from established treatment protocols by veterinary organizations. Moreover, the presence of stray dogs in rural areas and the outskirts of cities, along with incomplete strategies to address this issue, significantly contributes to the transmission and spread of the disease. These stray dogs act as reservoirs and amplifiers for the *Leishmania* parasite, further exacerbating the situation.

Furthermore, the increasing popularity of keeping pets in both urban and rural households has played a role in the rising number of cases. However, following-up measures in households to conduct clinical examinations and specific tests, including those for leishmaniasis, are insufficient, resulting in undiagnosed and untreated cases.

Advancements in diagnostic techniques, such as the simplified method of diagnosing visceral leishmaniasis in dogs using rapid strip tests and DAT, have facilitated easier and more efficient detection of the disease. This streamlined diagnostic approach has likely contributed to an increase in reported cases compared to previous decades.

Lastly, the escalation of VL among wild canids, including foxes and wolves, has led to the establishment of a sylvatic cycle for the *L. infantum* parasite. This cycle subsequently spills over into the domestic cycle, affecting the population of domestic dogs and further contributing to the overall rise in CanL.

The increasing incidence of CanL in the northwest and southern regions emphasizes the importance of strengthening veterinary services, implementing integrated control strategies, and raising awareness among the general population, dog owners, and healthcare professionals.

An important limitation of this study was sampling, particularly in passive cases, based on predominantly symptomatic patients' referrals. Likely, individuals with moderate or subclinical conditions did not refer. Furthermore, the same rationale accounts for the high VL seroprevalence for reference laboratory samples.

We recommend vector control, using ethical strategies for the reduction of stray dogs' population such as castrating of stray dogs, identifying suspect leashed dogs with periodic DAT and prompt treatment or surveillance of those found seropositive. Furthermore, we propose the significance of human case detection, efficient treatment, and targeted public health education, especially within VL endemic areas.

Conclusion

Mediterranean VL, a serious parasitic disease, is present in a number of different distinct regions across Iran. While examining HVL cases over the last decade revealed a declining trend, a similar trend was not seen for CanL, which means canines, which are crucial to the leishmaniosis cycle, are still of great importance. Comparing the genetic differences between canine- and human-isolated genotypes/strains of *L. infantum* using novel sequencing techniques such as NGS is recommended for future research.

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Conflict of interest

The authors have no conflicts of interest to declare for this study.

References

1. Alvar J, Vélez ID, Bern C, et al. Leishmaniasis worldwide and global estimates of its incidence. PLoS One. 2012;7(5):e35671.
2. WHO, 2022: Leishmaniasis. Available from: *https://www.who.int/news-room/fact-sheets/detail/leishmaniasis*.
3. Ready PD. Biology of phlebotomine sand flies as vectors of disease agents. Annu Rev Entomol. 2013;58:227-250.
4. Wang H, Naghavi M, Allen C, et al. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 2016;388(10053):1459-544.
5. Collaborators God, Bernabe E, Marcenes W, et al. Global, regional, and national levels and trends in burden of oral conditions from 1990 to 2017: a systematic analysis for the global burden of disease 2017 study. J Dent Res. 2020;99(4):362-73.
6. Badirzadeh A, Mohebali M, Asadgol Z, et al. The burden of leishmaniasis in Iran, acquired from the global burden of disease during 1990–2010. Asian Pac J Trop Dis. 2017;7(9):513-8.
7. Sharifi I, Khosravi A, Aflatoonian MR, et al. Cutaneous leishmaniasis situation analysis in the Islamic Republic of Iran in preparation for an elimination plan. Fron Public Health. 2023; 11 :1091709.
8. Edrissian GH, Nadim A, Alborzi A, Ardehali S. Visceral leishmaniasis: the Iranian experiences. Arch Iran Med. 1998; 1:22–26.
9. Sharifi I, Aflatonian MR, Daei Parizi MH, et al. Visceral Leishmaniasis in Southeastern Iran: A Narrative Review. Iran J Parasitol.2017; 12(1): 1-11.
10. Mohebali M, Edrissian GH, Nadim A, et al. Application of direct agglutination test (DAT) for the diagnosis and seroepidemiological studies of visceral leishmaniasis in Iran. Iran J Parasitol. 2006;1(1):15-25.
11. Rassi Y, Kaverizadeh F, Javadian E, Mohebali M. First report on natural promastigote infection of *Phlebotomus caucasicus* in a new focus of visceral leishmaniasis in North West of Iran. Iran J Publ Health. 2004;33(4):70-72 .
12. Akhoundi B, Mohebali M, Shojaee S, et al. Rapid detection of human and canine visceral leishmaniasis: assessment of a latex agglutination test based on the A2 antigen from amastigote forms of *Leishmania infantum*. Exp Parasitol. 2013; 133(3):307-13.
13. Akhoundi B, Mohebali M, Babakhan L, et al. Rapid detection of human *Leishmania infantum* infection: A comparative field study using the fast agglutination screening test and the direct agglutination test. Travel Med Infect Dis. 2010; 8(5):305-10.
14. Sarkari B, Rezaei Z, Mohebali M. Immunodiagnosis of Visceral Leishmaniasis: Current Status and Challenges: A Review Article. Iran J Parasitol. 2018; 13(3): 331-341.
15. Mohebali M, Keshavarz H, Shirmohammad S, et al. The diagnostic accuracy of direct agglutination test for serodiagnosis of human visceral leishmaniasis: a systematic review with meta-analysis. BMC Infect Dis. 2020; 20(1):946.
16. Mohebali M, Edrissian GH, Shirzadi M, et al. Integrated visceral leishmaniasis surveillance system in primary care for children in Meshkin-Shahr district, north-western Islamic Republic of Iran. East Mediterr Health J. 2010; 16 (10): 1050-1054.
17. Mohebali M. Visceral leishmaniasis in Iran: review of the epidemiological and clinical features. Iran J Parasitol. 2013; 8(3):348-358.
18. Rassi Y, Javadian E, Nadim A, et al. *Phlebotomus perfiliewi transcaucasicus*, a vector of *Leishmania*

infantum in northwestern Iran. J Med Entomol. 2009;46(5):1094-8.
19. Azizi K, Rassi Y, Javadian E, et al. First detection of *Leishmania infantum* in *Phlebotomus (Larroussius) major* (Diptera: Psychodidae) from Iran. J Med Entomol. 2008; 45(4):726-31.
20. Azizi K, Rassi Y, Javadian E, et al. *Phlebotomus (Paraphlebotomus) alexandri*: a probable vector of *Leishmania infantum* in Iran. Ann Trop Med Parasitol. 2006; 100(1):63-8.
21. Sahabi Z, Rashti MS, Nadim A, et al. A Preliminary report on the natural leptomonad Infection of *Phlebotomus major* in an endemic focus of visceral Leishmaniasis (VL) in Fars province, south of Iran. Iranian J Public Health. 1992; 21(1-4):87-93.
22. Rashti MS, Sahabi Z, Notash AK. *Phlebotomus (Larroussius) keshishiani Hchurenkova* 1936, another vector of visceral leishmaniasis in Iran. Iranian J Publ Health. 1995; 24(1-2):25-30.
23. Jahangir A, Akhoundi B, Mohebali M, et al. Seroepidemiological survey of human visceral leishmaniasis in Ilam province, west of Iran in 2013. Iran J Parasitol. 2015 ;10(1):56-61.
24. Heidari A, Mohebali M, Kabir K, et al. Visceral leishmaniasis in rural areas of Alborz province of Iran and implication to health policy. Korean J Parasitol. 2015; 53(4):379-83.
25. Khazaei S, Mohebali M, Akhoundi B, et al. Seroprevalence survey of visceral leishmaniasis among children up to 12 years old and domestic dogs in rural areas of Dehloran District, Ilam Province of west part of Iran, 2014. Novelty Biomed. 2017; 5(2):78-84.
26. Abbaszadeh-Afshar MJ, Mohebali M, et al. Seroepidemiological survey of visceral leishmaniasis among nomadic tribes of Kerman province, southeastern Iran: An observational study for implication to health policy. J Biostat Epidemiol. 2015;1(3/4):105-11.
27. Ebrahimzade-Parikhani H, Mohebali M, Zarei Z, Akhoundi B, Kakoei Z. Seroprevalence of visceral leishmaniasis in children up to 12 years old among nomadic tribes from rural areas of Pars Abad, northwestern Iran: an observational study in 2015. J Arthropod Borne Dis. 2017; 11(2):331-337.
28. Shirmohammad S, Mohebali M, Ghalehbin BM,et al. Human visceral leishmaniasis: Seroprevalence survey of asymptomatic adults in an endemic area of Northwestern Iran. J Biostat Epidemiol. 2016;2(3):136-42.
29. Afshar MJA, Sharifi I, Bamorovat M, et al. Canine visceral leishmaniasis; A seroepidemiological survey in Jiroft district, southern Kerman province, southeastern Iran in 2015. Iran J Parasitol. 2018; 13(1):67-71.
30. Behniafar H, Moin-Vaziri V, Mohebali M, et al. Visceral leishmaniasis among children in an endemic area of northwestern Iran between 2016 and 2017: An epidemiological study. Asian Pac J Trop Med. 2019; 12(7):306-314.
31. Ghodrati S, Akhoundi B, Mohebali M, et al. A Sero-Epidemiological Study on Visceral Leishmaniasis among Volunteer Children and Adults in Rural Areas of Shahroud, Iran 2018–2019. J Arthropod Borne Dis. 2022; 16(3):217–224.
32. Shad IAN, Mahmoudi MR, Mohebali M, et al. Seroepidemiological Study of Visceral Leishmaniasis (Kala-azar) in Children under 12 Years Old in North of Iran: An Observational Study in 2019-2020. Iran J Parasitol. 2022; 17(3):317-24.
33. Sedaghatmanesh K, Khazan H, Akhoundi B, Khazaei S, Kakooei Z, Mohebali M. Seroprevalence of Visceral Leishmaniasis in Children Up To 12 Years Old of Rural Areas from Kermanshah Province, Western Part of Iran. Iran J Parasitol. 2023; 18(1):85-92.
34. Bamorovat M, Sharifi I, Mohammadi MA, et al. Canine visceral leishmaniasis in Kerman, southeast of Iran: a seroepidemiological, histopathological and molecular study. Iran J Parasitol. 2014; 9(3):342-349.
35. Tabatabaie F, Nasirikaleybar Y, Mohebali M, et al. Serological and molecular survey of zoonotic visceral leishmaniasis in stray dogs (*Canis familiaris*) from an endemic focus in Meshkin-Shahr district in Ardabil province, Iran. J Vector Borne Dis. 2021; 58(3):213-218.
36. Farahmand M, Nahrevanian H, Khalaj V, et al. Assessment of recombinant A2-Latex Agglutination Test (RA2-LAT) and RA2-ELISA for detection of canine visceral Leishmaniasis: a comparative field study with direct agglutination test in Northwestern Iran. Iran J Parasitol. 2018; 13(2):172-179.
37. Heidari A, Mohebali M, Vahed M, et al. Molecular and Seroepidemiological survey of visceral leishmaniasis in owned dogs (*Canis*

familiaris) in new foci of rural areas of Alborz Province, Central Part of Iran: A cross-sectional study in 2017. J Arthropod Borne Dis. 2020; 14(1):38-46.
38. Soleimani A, Mohebali M, Gholizadeh S, et al. Molecular and serological evaluation of visceral leishmaniasis in domestic dogs and cats in Maragheh County, north-west of Iran, 2018–2021. Vet Med Sci. 2022; 8(5):1898-1903.
39. Mohebali M, Malmasi A, Khodabakhsh M, et al. Feline leishmaniosis due to *Leishmania infantum* in Northwest Iran: The role of cats in endemic areas of visceral leishmaniosis. Vet Parasitol Reg Stud Reports. 2017; 9:13-6.
40. Fatollahzadeh M, Khanmohammadi M, Bazmani A, et al. Survey of feline visceral leishmaniasis in Azarshahr area, North West of Iran, 2013. J Parasit Dis. 2016; 40(3):683-7.
41. Mohebali M, Arzamani K, Zarei Z, et al. Canine visceral leishmaniasis in wild canines (fox, jackal, and wolf) in northeastern Iran using parasitological, serological, and molecular methods. J Arthropod Borne Dis. 2016; 10(4):538-545.
42. Nadim A, Javadian E, Tahvildar–Bidruni G, Mottaghi M. Epidemiological aspects of kala-azar in Meshkin shar, Iran: investigation on vectors. Iranian J Publ Health. 1992; 21(1-4):61-72.
43. Rassi Y, Javadian E, Nadim A, Zahraeii AR, Vatandoost H. *Phlebotomus (Larroussius) kandelakii*, the principal and proven vector of visceral leishmaniasis in north west of Iran. Pak J Biol Sci. 2005; 80:1–5.
44. Rassi Y, Abai M, Oshaghi M, et al. First detection of *Leishmania infantum* in *Phlebotomus kandelakii* using molecular methods in north-eastern Islamic Republic of Iran. East Mediterr Health J. 2012; 18 (4), 387-392.
45. Dehkordi AS, Rassi Y, Oshaghi M, et al. Molecular detection of *Leishmania infantum* in naturally infected *Phlebotomus perfiliewi transcaucasicus* in Bilesavar district, northwestern Iran. Iran J Arthropod Borne Dis. 2011; 5(1):20-27.
46. Oshaghi MA, Ravasan NM, Hide M, et al. *Phlebotomus perfiliewi* transcaucasicus is circulating both *Leishmania donovani* and *L. infantum* in northwest Iran. Exp Parasitol. 2009; 123(3):218-25.
47. Parvizi P, Mazloumi-Gavgani A, Davies C, Courtenay O, Ready P. Two *Leishmania* species circulating in the Kaleybar focus of infantile visceral leishmaniasis, northwest Iran: implications for deltamethrin dog collar intervention. Trans R Soc Trop Med Hyg. 2008; 102(9):891-7.
48. Oshaghi MA, Rassi Y, Hazratian T, Fallah E, Rafizadeh S. Natural infection of wild caught Phlebotomus tobbi to *Leishmania infantum* in East Azerbaijan province, northwestern Iran. J Vector Borne Dis. 2013; 50(1):24-29.
49. Rassi Y, Dehkordi AS, Oshaghi MA, et al. First report on natural infection of the *Phlebotomus tobbi* by *Leishmania infantum* in northwestern Iran. Exp Parasitol. 2012; 131(3):344-9.
50. Bahrami A, Rassi Y, Maleki N, et al. *Leishmania infantum* DNA detection in *Phlebotomus tobbi* in a new northern focus of visceral leishmaniasis in Iran. Asian Pac J Trop Dis. 2014; 4(2):110-114.
51. Javadian E, Mesghali A, Nadim A. Natural leptomonad infection of sand flies with its first occurrence in *P. alexandri* in Khuzistan Province, Iran. Coll Int CNRS. 1977; 239:203-5.
52. Rassi Y, Karami H, Abai MR, et al. First detection of *Leishmania infantum* DNA in wild caught *Phlebotomus papatasi* in endemic focus of cutaneous leishmaniasis, South of Iran. Asian Pac J Trop Biomed. 2013; 3(10):825-9.
53. Rostamian M, Bashiri H, Yousefinejad V, et al. Prevalence of human visceral leishmaniasis in Iran: A systematic review and meta-analysis. Comp Immunol Microbiol Infect Dis. 2021; 75:101604.
54. Mondiale de la Santé O, WHO. Weekly Epidemiological Record, 2022, vol. 97, 45 [full issue]. Weekly Epidemiological Record= Relevé épidémiologique hebdomadaire. 2022; 97(45):575-90.
55. Ready PD. Epidemiology of visceral leishmaniasis. Clin Epidemiol. 2014; 6:147-154.
56. Marcondes M, Day MJ. Current status and management of canine leishmaniasis in Latin America. Res Vet Sci. 2019; 123:261-72.
57. Davies C, Gavgani AM. Age, acquired immunity and the risk of visceral leishmaniasis: a prospective study in Iran. Parasitology. 1999; 119(Pt 3):247-57.
58. Shokri A, Fakhar M, Teshnizi SH. Canine visceral leishmaniasis in Iran: A systematic

review and meta-analysis. Acta Trop. 2017; 165:76-89.
59. Mohebali M, Moradi-Asl E, Rassi Y. Geographic distribution and spatial analysis of *Leishmania infantum* infection in domestic and wild animal reservoir hosts of zoonotic visceral leishmaniasis in Iran: A systematic review. J Vector Borne Dis. 2018; 55(3):173-183.
60. WHO. Global vector control response 2017-2030. Geneva: World Health Organization; 2017.
61. Githeko AK, Lindsay SW, Confalonieri UE, Patz JA. Climate change and vector-borne diseases: a regional analysis. Bulletin of the World Health Organization. 2000; 78(9):1136-47.
62. Wamai RG, Kahn J, McGloin J, Ziaggi G. Visceral leishmaniasis: a global overview. J Glob Health. 2020; 14;2(1).
63. Patiño LH, Castillo-Castañeda AC, Muñoz M, et al. Development of an amplicon-based next-generation sequencing protocol to identify *Leishmania* species and other trypanosomatids in leishmaniasis endemic areas. Microbiol Spectr. 2021;9(2):e0065221.
64. de Arruda RMF, Cardoso DT, Teixeira-Neto RG, et al. Space-time analysis of the incidence of human visceral leishmaniasis (VL) and prevalence of canine VL in a municipality of southeastern Brazil: Identification of priority areas for surveillance and control. Acta Trop. 2019; 197:105052-105052.
65. Ciaramella P, Oliva Gd, De Luna R, et al. A retrospective clinical study of canine leishmaniasis in 150 dogs naturally infected by *Leishmania infantum*. Vet Rec. 1997; 141(21):539-43.
66. WHO. Report of the Second WHO Meeting on Emerging Infectious Diseases, Geneva, Switzerland, and 12-13 January 1995. World Health Organization; 1995.
67. Saini I, Joshi J, Kaur S. Unwelcome prevalence of leishmaniasis with several other infectious diseases. Int Immunopharmacol. 2022; 110:109059.
68. Kantzanou M, Karalexi MA, Theodoridou K, et al. Prevalence of visceral leishmaniasis among people with HIV: a systematic review and meta-analysis. Eur J Clin Microbiol Infect Dis. 2023; 42(1):1-12.
69. Medrano FJ, Hernández-Quero J, Jiménez E, et al. Visceral leishmaniasis in HIV-1-infected individuals: a common opportunistic infection in Spain? AIDS. 1992; 6(12):1499-503.
70. Rezaei Z, Sarkari B, Dehghani M, Layegh Gigloo A, Afrashteh M. High frequency of subclinical *Leishmania* infection among HIV-infected patients living in the endemic areas of visceral leishmaniasis in Fars province, southern Iran. Parasitol Res. 2018; 117(8):2591-5.
71. Shafiei R, Mohebali M, Akhoundi B, et al. Emergence of co-infection of visceral leishmaniasis in HIV-positive patients in northeast Iran: A preliminary study. Travel Med Infect Dis. 2014; 12(2):173-8.