

Original Article



Serosurvey of Visceral Leishmaniasis Among Drug Users in Fars Province, Southern Iran: A Cross-sectional Analysis

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Article history:

Received: April 6, 2025

Revised: July 30, 2025

Accepted: August 3, 2025

ePublished: February 23, 2026

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Abstract

Background: Drug addiction is known to compromise the immune system, thereby increasing susceptibility to infectious diseases. However, the status of visceral leishmaniasis (VL) among drug users, a high-risk group, has not been well understood yet. Therefore, this study aimed to explore the seroepidemiology of VL among drug users in Fars Province, southern Iran.

Methods: This cross-sectional study analyzed 1,400 serum samples from drug users in Fars Province for anti-*Leishmania* antibodies using a homemade enzyme-linked immunosorbent assay method. The data collected during sampling were documented in a questionnaire and analyzed with SPSS (version 27).

Results: The mean age of the participants was 44.59 (\pm 12.50) years, with 52.7% aged between 40 and 50 years. Anti-*Leishmania* antibodies were detected in the sera of 27 (1.9%) of the subjects, predominantly in males (92.6%). No significant gender difference was observed in the presence of antibodies. Additionally, seroprevalence did not noticeably differ between injecting and non-injecting drug users. Eventually, the majority of seropositive individuals were under the age of 20 years (18.2%, n = 11), highlighting a higher burden among younger participants.

Conclusion: The findings revealed a significant prevalence of VL among drug users in Fars Province, indicating the importance of tailored public health initiatives and additional studies to support this at-risk group.

Keywords: Visceral leishmaniasis, Drug users, Fars province, Southern Iran



Please cite this article as follows: Dehghani M, Zarrabi M, Shahriarirad S, Rohampour S, Shayan G, Omidian M, et al. Serosurvey of visceral leishmaniasis among drug users in Fars province, southern Iran: a cross-sectional analysis. *Avicenna J Clin Microbiol Infect.* 2026;13(1):37-41. doi:10.34172/ajcmi.3638

Introduction

Visceral leishmaniasis (VL) is a neglected protozoan disease that is the most severe form of leishmaniasis and can be fatal if left untreated. VL is transmitted by the bite of infected female sandflies, with the *Leishmania* (*L.*) *donovani* and *L. infantum/chagasi* complex parasites being the primary etiologic agents. In Iran and the Middle East, *L. infantum* is the predominant cause, mainly impacting children in areas where the disease is endemic and dogs serve as the principal reservoir (1-5).

Opioid abuse is a major public health threat in Iran and its neighbors. Opioids rank among the most widely used drugs across Asia and Europe, with heroin leading global consumption. Although opioid use is illegal in Iran, it is still prevalent. This has led the government to expand addiction treatment programs and open additional

rehabilitation centers (6,7). Despite their official illegality and the harsh penalties associated with their use and trade, opiate trafficking and consumption remain serious public health challenges in the country. In response to this ongoing crisis, the government has implemented various measures, including providing support and treatment for addicts and establishing rehabilitation centers to initiate preventive interventions.

Drug-induced immunosuppression, particularly in intravenous drug users (IVDUs), compromises cellular immunity and may facilitate *Leishmania* infection. Several studies report a incidence of higher VL among IVDUs, likely driven by both altered immune responses and overlapping risk behaviors (8-10).

Sharing needles among IVDUs has been suggested as a possible alternative, artificial (i.e., laboratory-mediated



transfusion or needle sharing), and anthroponotic transmission route for *Leishmania* infection. The reported cases of VL are more common among acquired immunodeficiency syndrome patients who are or were IVDUs compared to those who contracted human immunodeficiency virus (HIV)-1 through other means (11). VL poses a significant public health challenge in Iran (1,3,4,12,13). While VL primarily affects vulnerable populations, certain high-risk groups (e.g., individuals with substance use disorders) may face an elevated risk due to environmental and behavioral factors (14,15). Drug addiction often leads to compromised immune systems, substandard living conditions, and engagement in risky behaviors, all increasing susceptibility to infections like VL (16). Injecting drug users, in particular, are at heightened risk due to practices such as needle sharing, which facilitates the transmission of bloodborne pathogens, and frequent exposure to environments conducive to sandfly proliferation. Additionally, malnutrition and co-infections, commonly observed among this demographic, can exacerbate the severity of VL (16).

Fars Province in southern Iran is a known VL endemic area (annual incidence: ~2.5 per 100,000) with rising rates of opioid use (estimated 3.8% adult prevalence) (4,5,10,17). Despite its public health significance, research specifically focusing on VL among drug users in Iran remains scarce, even though the disease represents a significant health challenge in the region. To the best of our knowledge, no seroepidemiological data have so far been found on VL prevalence among drug-using groups in Fars province, a region endemic for *L. infantum*. Accordingly, the current study aims to bridge this knowledge gap by examining the seroprevalence of VL among drug users in Fars Province, southern Iran, through a cross-sectional analysis. Given the dual burden of opiate-related immunosuppression and vector-borne exposure, our study fills a critical gap by providing the first cross-sectional serosurvey in this vulnerable group. It is hoped that the results provide valuable insights into the prevalence and associated risk factors within this population, thereby facilitating evidence-based policymaking and efficient resource distribution.

Methods

Study Area and Sampling

This cross-sectional study was conducted between June 2023 and April 2024, involving 1,400 drug users from Fars Province, southern Iran (coordinates: N 29° 6' 15.7727", E 53° 2' 45.2148"). The inclusion criteria comprised adults (≥ 18 years) with self-reported drug use registered at harm-reduction centers in Fars province. On the other hand, the exclusion criteria included individuals unwilling to donate a blood sample, unwilling to participate in the study, or having insufficient serum volume for testing.

Aseptic 5 mL blood samples were collected from each participant and carefully transported in cold storage to the Parasitology and Mycology Department at Shiraz

University of Medical Sciences, Shiraz, Iran. The serum was extracted using a centrifuge and stored at -20°C until further analysis.

Preparation of *Leishmania* Antigen

The crude soluble antigen for enzyme-linked immunosorbent assay (ELISA) was derived from a reference human strain of *L. infantum*. Promastigotes were grown in the RPMI 1640 medium enriched with 10% inactivated fetal bovine serum at 26°C . After large-scale cultivation, the promastigote pellet was collected through centrifugation, rinsed three times with phosphate-buffered saline (PBS), and subjected to five freeze-thaw cycles followed by sonication for lysis. The protein concentration was measured using the Bradford assay. The prepared antigen extracts were aliquoted and stored at -20°C , minimizing freeze-thaw cycles.

Enzyme-Linked Immunosorbent Assay for Detection of Anti-*Leishmania* Antibodies

An ELISA system, using *L. infantum* crude antigen, was used to detect anti-*Leishmania* antibodies in the serum samples. This ELISA underwent rigorous validation in our two prior studies (18,19). In these studies, the assay demonstrated 83.6% sensitivity and 90.5% specificity when compared to the direct agglutination test or indirect immunofluorescence (12,17,20). Following these validations, our research group applied this standardized assay in four subsequent seroprevalence investigations, confirming its reproducibility and accuracy across diverse populations.

Fifteen flat-bottom 96-well microplates were coated with 100 μL of purified antigen at a concentration of 5 $\mu\text{g}/\text{mL}$, dissolved in 0.1 M carbonate/bicarbonate buffer (pH: 9.6), and incubated overnight at 4°C . An antigen concentration of 5 $\mu\text{g}/\text{mL}$ was selected based on an ELISA checkerboard and our prior optimization studies, demonstrating maximal sensitivity and specificity.

Excess antigens were removed by washing the plates five times with PBS containing 0.05% Tween 20 (PBST, pH: 7.4). Blocking was performed by adding 200 μL of 3% nonfat skimmed milk to PBST for 2 hours at room temperature. After additional washing, the serum samples, which were diluted at 1:100 in PBST, were introduced into the wells and then incubated for 1 hour at room temperature. Subsequently, 100 μL of horseradish peroxidase-conjugated goat anti-human immunoglobulin G (1:4000 dilution, Sigma, USA) was added to the wells and incubated for 1.5 hours at room temperature, followed by another washing step. Next, the substrate solution (100 μL per well; 0.4 mg/mL o-phenylenediamine dihydrochloride, 0.3% H_2O_2 in 0.1 M citrate buffer, pH: 5) was applied and incubated for 20 minutes. The reaction was stopped with 1 N H_2SO_4 , and absorbance at 490 nm was measured using a microplate reader. Positive sera from confirmed VL cases were included on each plate, with the cutoff value established at 2 standard deviations

above the mean absorbance of control samples.

Statistical Analysis

Demographic data and test results of the participants were entered into SPSS software (version 27), and the chi-square test was utilized to evaluate associations between *Leishmania* seropositivity and demographic variables.

Results

Study Overview

This study involved 1,400 individuals with a mean age of 44.59 ± 12.50 years (range: 17–73). The population was predominantly male (92.8%, $n = 1,299$), with females accounting for 7.2% ($n = 101$). Among the participants, 1.9% ($n = 27$) tested positive for VL, with a notable proportion residing in Shiraz (25.9%, $n = 7$). A statistically meaningful association was identified between VL and the place of residence ($P < 0.05$).

Seropositive Population Characteristics

Among individuals positive for anti-*Leishmania* antibodies, 92.6% ($n = 25$) were male, and 7.4% ($n = 2$) were female; however, this gender-based difference was not statistically significant ($P > 0.05$). In contrast, the majority of seropositive individuals were under the age of 20 years (18.2%, $n = 11$), and this age-related difference was statistically significant ($P < 0.05$).

Drug Use and Leishmaniasis

Most subjects reported using non-injection routes as their primary method of drug use (87.2%, $n = 1,221$). However, the method of drug abuse did not yield statistically significant findings ($P > 0.05$). Table 1 outlines the socio-demographic profiles of drug users, along with their corresponding rates of *Leishmania* seropositivity, in Fars Province, southern Iran.

Table 1. Traits of Drug Users and Their Corresponding Rates of Seropositivity for *Leishmania* in Fars Province, Southern Iran

Variables	Frequency (Percent)	Positive for anti- <i>Leishmania</i> Antibodies (Percent)	P value
Gender			
Male	1,299 (92.8%)	25 (92.6%)	0.77
Female	101 (7.2%)	2 (7.4%)	
Age			
<20	11 (0.78%)	2 (18.2%)	0.016*
20-39	525 (37.5%)	12 (2.3%)	
40-50	738 (52.7%)	13 (1.8%)	
>50	126 (9%)	0 (0%)	
Residential location			
Urban	1,167 (83.4%)	25 (92.6%)	0.013*
Rural	233 (16.6%)	2 (7.4%)	
Drug use method			
Drug injection	179 (12.8%)	4 (14.8%)	0.769
Other means	1,221 (87.2%)	23 (85.2%)	

Discussion

VL, caused by the protozoa of the *Leishmania* genus, is a severe parasitic disease predominantly affecting individuals in tropical and subtropical regions (21–23). Among drug users, particularly those who inject drugs, the risk of VL transmission is heightened due to shared needles and syringes, which can serve as vectors for the parasite (14,24–26). This mode of transmission underscores the importance of addressing VL within this vulnerable population.

The current study investigated the seroepidemiology of VL among drug users in Fars Province, southern Iran. The findings provided valuable insights into the prevalence and distribution of anti-*Leishmania* antibodies in this high-risk population, contributing to the broader understanding of VL epidemiology in endemic regions. Based on the results, the overall seroprevalence of anti-*Leishmania* antibodies among drug users was 1.9%. The prevalence of anti-*Leishmania* antibodies was significantly higher among participants under 20 years old compared to other age groups, suggesting that younger drug users may be more vulnerable to VL infection, potentially due to behavioral factors and weaker immune responses.

The study also examined the relationship between injection drug use and VL seroprevalence, where the seroprevalence rates were 1.88% and 2.23%, respectively, with no statistically significant difference between the two groups. The results demonstrated that both the small number of seropositive cases and potential behavioral homogeneity (e.g., similar exposure to sand flies across subgroups) likely contributed to this nonsignificant finding.

While injection drug use is a known risk factor for infectious diseases, it did not appear to significantly influence VL infection risk in this population. Nonetheless, factors such as malnutrition and co-infections, which are common among drug users, may contribute to VL susceptibility.

The epidemiology of VL among drug users is multifaceted, shaped by a number of factors (e.g., socioeconomic status, healthcare accessibility, and concurrent infections like HIV). The coexistence of HIV and VL is especially alarming, as it intensifies the immunosuppressive impact of both conditions, resulting in increased morbidity and mortality (8). Drug users may face additional barriers to diagnosis and treatment (e.g., stigma and limited healthcare resources) in endemic regions. Moreover, the co-infection of *Leishmania* and HIV has not only significantly increased the transmission of parasites via sandflies from immunocompromised individuals to others but has also likely facilitated artificial transmission among immunocompromised IVDUs through shared needles (26,27).

Sharing needles among IVDUs has been suggested as creating an alternative, artificial, and anthroponotic cycle for *Leishmania* transmission (27). Cruz et al evaluated discarded syringes from IVDUs using the polymerase

chain reaction method and found that in southern Madrid, Spain, 65 out of 125 syringes (52%) tested positive for *Leishmania* spp. Similarly, in southwestern Madrid, 52 out of 154 syringes (34%) showed the presence of the parasite (14). Likewise, Pineda et al investigated *Leishmania* infection markers in 93 IVDUs and 77 individuals who did not use intravenous drugs using *Leishmania* skin testing and serum antibody detection. Their results revealed that 24 IVDUs were skin-positive in comparison with 10 non-users of intravenous drugs. Additionally, *Leishmania* seropositivity was identified in 3 of 11 active IVDUs and in 3 of 82 former injectors (26). These findings suggest that needle sharing plays a role in the transmission of the parasite and highlight the need for preventive measures.

Preventive measures (e.g., needle exchange programs and education campaigns) can play a pivotal role in reducing VL transmission among drug users. Moreover, tackling the social determinants of health, including poverty and unstable housing, can help reduce the risk factors linked to VL. Collaborative efforts among public health authorities, healthcare providers, and community organizations are crucial to effectively combat VL in this population.

The identification of a 1.9% seroprevalence rate among drug users in the current study emphasizes the need for targeted screening and intervention programs for this high-risk population. Public health efforts should focus on younger individuals. Furthermore, the absence of considerable differences in seroprevalence based on gender or drug use method suggests that interventions should be inclusive and address the broader determinants of health among drug users. Future studies should explore factors driving higher seroprevalence among younger drug users and specific geographic areas. Longitudinal research can offer insights into the temporal dynamics of VL infection and its associated risk factors. Incorporating molecular diagnostic techniques (e.g., polymerase chain reaction) can enhance detection accuracy and identify asymptomatic carriers contributing to the transmission cycle.

Conclusion

In this large-scale serosurvey of 1,400 drug users in Fars Province, a 1.9% overall seroprevalence of VL was identified, with individuals under 20 years exhibiting the highest burden. This age distribution mirrors national VL trends in Iran, where children and adolescents consistently demonstrate higher disease incidence than older groups. These findings underscore the need for age-specific, drug-user-focused public health strategies (e.g., youth-targeted screening campaigns, school-based awareness programs, and integrated VL/HIV outreach within harm-reduction services) to interrupt transmission and mitigate risk. By tailoring interventions to the distinct exposure patterns and immunological vulnerabilities of younger drug users, health authorities can enhance disease control efforts and

ultimately reduce the VL burden in endemic regions.

Despite the strengths of our large-scale serosurvey, the cross-sectional design, convenience sampling, and lack of HIV and CD4 count data warrant cautious interpretation and highlight the need for longitudinal, multicenter studies.

Acknowledgments

The study was a part of the results of an MD dissertation submitted to Shiraz University of Medical Sciences by Dr. Mahshid Dehghani.

Authors' Contribution

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Competing Interests

The authors declare no competing interests.

Ethical Approval

Ethical approval for the study was obtained from the Ethics Committee of Shiraz University of Medical Sciences (IR.SUMS.MED.REC.1403.403). In addition, informed consent was obtained from all participants involved in the study.

Funding

The financial support for the research was provided by the Vice-Chancellor's Office of Shiraz University of Medical Sciences (Grant No. 31002).

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