Original Article

Human Visceral Leishmaniasis: a Serological Survey in Rural Areas of Dashti District of Bushehr Province, Southern Iran

Mohammad Gorgipour[1], Mehdi Mohebali[2,3], Behnaz Akhoundi[2], Mohammad javad Abbaszadeh Afshar[2], Bahram Kazemi[5], Sasan Khazaei[1], Eznollah Azargashsb[5], Hooshang Khazan[1*]

[1] Department of Medical Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[3] Center for Research of Endemic Parasites of Iran (CREPI), Tehran University of Medical Sciences, Tehran, Iran
[4] Department of Biotechnology, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[5] Department of Social Medicine, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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Abstract

Background: Visceral leishmaniasis (VL) or kala-azar is a parasitic disease caused by the species of Leishmania donovani complex. Mediterranean type of the disease is endemic in some parts of Iran and more than 95% of cases were reported in children up to 12 years of age. This study was performed to determine the seroprevalence of VL in the rural areas of the Dashti district from Bushehr province.

Materials and Methods: In this cross-sectional study, a randomized cluster sampling method was used for the collection of blood samples from children up to 12 years old from rural areas of Dashti district. Before sampling; a questionnaire was filled out for each case. All the collected blood samples were examined after the serum separating by Direct Agglutination Test (DAT) for detection of anti-*Leishmania infantum* antibodies. The cutoff titers of ≥1: 3200 with specific clinical features were supposed to be considered as VL.

Results: Altogether, 24 out of 1221 (1.96%) blood samples showed titers between 1:800 and 1:1600 which considered as suspicious cases. None of the suspicious cases had a history of kala-azar. None of 1221 collected blood samples showed anti *Leishmania infantum* (*L. infantum*) at titer ≥1:3200.

Conclusion: This study confirms the circulation of *L. infantum* in Dashti district and highlights the sporadic pattern of VL in the studied areas which necessitates the surveillance system to be monitored by health authorities.

Keywords: Visceral leishmaniasis, Direct Agglutination Test, human, Iran

***Corresponding Author:** Hooshang Khazan, Department of Medical Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Email: Khazan_h36@yahoo.co.in

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Introduction

Visceral leishmaniasis (VL) so-called kala-azar caused by the species of *Leishmania donovani* complex is a systemic parasitic disease which is transmitted by Phlebotominae sand flies. *Canis familiaris* are principal VL reservoir hosts in Mediterranean type of VL[1,2]. This disease is responsible about 500,000 new cases each year and about 59,000 deaths annually. Approximately, 100-300 new cases of VL are reported in Iran every year[2,3]. VL is common (over 98%) among children

under 12 years old in different endemic foci in Iran and adult cases frequently present with subclinical and asymptomatic forms in endemic regions[4,5]. Kala-azar is characterized by fever, weight loss, mucosal ulcers, fatigue, anemia and substantial hyperplasia of the liver and spleen and even death in most of untreated cases[1,6]. The first report of VL in Iran was from the Mazandaran province in 1949[7]. At least six endemic foci of VL have been detected in some areas of Ardabil, East Azerbaijan, Fars, Bushehr and recently from Qom and northern Khorasan provinces[2,8]. Other parts of Iran are considered as sporadic areas for VL. In this study, Direct Agglutination Test (DAT) was used as sero-diagnostic tool since it is a valid, cost effective, sensitive/specific, and user friendly test[2,9]. From 1991 to 1997, 92 cases of kala azar were diagnosed in Dashti and Dashtestan district of Bushehr province. These districts are near southern part of Fars province, areas known to be endemic foci of kala-azar in Iran[10]. The current investigation was performed to determine the sero-prevalence of VL in Dashti district of Bushehr province.

Methods

Study area: Dashti district with 77,530 populations and 50,028 square kilometers has 4 urban centers and 93 villages. This district is situated on the southern slope of the Zagros range of mountains, nearly the south of the Bushehr province (Figure1). The study area has a tropical climate with an average altitude of 65 meters above the sea level (Available at: https://en.wikipedia.org/wi-ki/Jiroft_County).

Blood Sampling: This cross-sectional study was carried out as a descriptive survey during March 2014 to February 2015 and a randomized cluster sampling method was used for the sample collection. A questionnaire was completed for each individual to recording demographic characteristics such as age, gender, village, symptoms, history of VL and close contact with the dog. Sixteen villages (cluster) from 93 villages in four geographical zones of Dashti district were randomly selected. Blood samples (0.5-1 ml) were collected from 1221 children up to 12 years old in tubes containing heparin as anticoagulant and processed them 4-10 h after collection. Serum samples were separated by centrifugation at 800 ×g for 5 minutes and stored at −20°C for further serological examination.

Direct Agglutination Test: All the collected serum samples were examined by DAT. In order to determine the *Leishmania*-specific antibodies titers a procedure described by Harith *et al*, was used[11]. Initially, for screening purposes, two dilutions of 1:800 and 1:3200 were provided and tested. The samples that were positive with titers of 1:800 were diluted up to 1:102400 in a V-shaped micro-titer plate into a dilution fluid containing 0.9% saline and 0.78% 2-mercaptoethanol. An equal volume (50 µl) of antigen suspension was added to each well. The results were read after 18-24 hours' incubation in a wet room at room temperature. The highest dilution at which agglutination was still visible in comparison with positive and negative control titers was defined as the sample titer. Compact blue dots were scored as negative and large diffuse blue mats as positive. The known negative and positive controls were tested in each plate daily. Titers of ≥1: 3200 were considered as seropositive cases[8, 12]. The cutoff was based on previous studies[4,11,13-15]. DAT Leishmania antigen was prepared in the protozoology unit of the School of Public Health, Tehran University of Medical Sciences.

Statistical analysis: Chi-squared and Fisher exact tests were used to compare Sero-prevalence values relative to gender and age groups. Analyses were performed with SPSS (version 18), with a probability p value of <0.05 considered as statistically significant.

Results

Totally, 1221 blood samples collected from children up to 12 years old by Dashti district from Bushehr province [634 (52%) for males and 587 (48%) from females]. After screening, 24 cases (1.96%) were shown *L. infantum* specific antibodies at titers 1:800. Five (0.4%) out of 24 cases were diagnosed as suspicious cases with titers of 1:1600 (Table 1). Also, none of the evaluated subjects were found to be DAT positive (at titers ≥1: 3200). There was no significant difference between male and female at 1:800 and 1: 1600 titers of anti-*Leishmania* specific antibodies (*P>0.05*). Although, the number of human cases with 1:800 and 1:1600 titers in males were higher than females. Frequency of anti-*Leishmania* antibody titers

Figure 1. The map showing location of Dashti district as a subpart of Bushehr province in Iran used as target population of the study.

Table 1: Seroprevalence of human visceral Leishmaniasis by Direct Agglutination Test with anti-*Leishmania infantum* antibodies based on gender and age groups in Dashti district, 2014.

parameters	**No. of sera**	**Antibody Titer**				**Total**	
		1:800		1:1600			
		No. of sera	%	No. of sera	%	No.of sera	%
Gender							
Male	634	13	2	5	0.8	18	2.8
Female	587	6	1	0	0	6	1
Age (yr)							
≤ 4	364	6	1.6	1	0.3	7	1.92
5-8	462	4	0.8	1	0.2	5	1
9-12	395	9	2.3	3	0.7	12	3
Total	1221	19	1.6	5	0.4	24	1.96

Table 2: Distribution of target population for detection of seroprevalence of human visceral leishmaniasis according to gender and age groups in Dashti district, 2014.

Age (yr)	**Male**		**Female**		**Total**	
	No. of sera	%	No. of sera	%	No. of sera	%
≤ 4	191	52.4	173	47.6	364	30
5-8	254	55	208	45	462	38
9-12	189	48	206	52	395	32
Total	634	52	587	48	1221	100

with DAT according to the age groups is indicated in Table 1. Age and gender distribution of samples are shown in Table (2). The highest percentage of seropositivity was observed in age groups of 9-12 years old (3%). Meanwhile, re-sampling of all suspicious cases and testing they did not show

increased titers of *L. infantum* specific antibodies. None suspicious cases had clinical signs.

Discussion

Bushehr province is one of the endemic parts of visceral leishmaniasis in Iran[12]. In this province a high number of cases of kala-azar were recorded during previous decades. From 1991 to 1997, 92 cases of kala-azar were diagnosed in the Dashti and Dashtestan districts in Bushehr. These districts are near south of Fars province, areas known to be endemic foci of kala-azar in Iran[10]. Since no serological study had been performed in last decade in this province, the present study was carried out to evaluate the epidemiological aspects of VL in this area. Because of its highly sensitive and specific results for detection of *L. infantum* infection in humans, DAT analysis was applied as a main tool of this survey[8].

The sensitivity and specificity of this method varies in different studies between 90–100% and 72–100% respectively[9]. Of the 1221 samples, 24 cases (1.96%) showed titers between 1: 800 and 1: 1600 by DAT, although none of those were found DAT positive (at titers ≥1:3200). Most cases that showed titers between 1: 800 and 1: 1600 were detected in children of 9-12 years old (3%). Based on our results, currently, VL occurs with sporadic pattern in Dashti district of Bushehr province. However, according to a previous study made by Mohebali *et al*, in 2001, Dashti and Dashtastan were reported as endemic areas of kala-azar where They found 3.4% of sero-positive cases in Dashti and Dashtestan districts[10]. These differences seem to be associated with a proper control of the disease over the years by health authorities of the strict or differences in method of sampling, number of population and the presence or absence of clinical symptoms. In 2006, Asgari et al. found 1.86% of sero-positivity in Qashqai tribes in south of Fars province. These people travel with their flocks each year from the summer highland pastures “Yailaq” in south of Fars province to winter quarters, on lower (and warmer) lands “Qishlaq” in Dashti and Dashtestan districts in Bushehr province. It seems that *L.infantum* infection among people of Dashti district is in association with the presence of Qashqai tribes in this district in part of year. In Qom province, Fakhar et al. found 1.7% of sero-positivity in 8 villages of Ghahan in which three over seven seropositive cases had a previous history of VL[16]. In our study None of the cases with 1: 800 and 1: 1600 titers of anti-*Leishmania*-specific antibodies presented a history of kala-azar.

Conclusion

This study confirms the circulation of *L. infantum* in Dashti district and highlights the sporadic pattern of VL in the studied areas. Further investigations regarding the sand file's fauna and wild canines as reservoir hosts of the disease are recommended.

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