

## Original Article

## Seroepidemiological survey of *Visceral leishmaniasis* among nomadic tribes of Kerman Province, Southeastern Iran: An observational study for implication to health policy

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## ABSTRACT

**Background & Aim:** Visceral leishmaniasis (VL) or kala-azar is a parasitic disease caused by the species of *Leishmania donovani* complex. Mediterranean type of disease is endemic in some parts of Iran and more than 95% of seropositivity cases were reported in children up to 12 years of age. A cross-sectional study was conducted to determine the seroprevalence of VL in nomadic tribe's population of the Kerman Province.

**Methods & Materials:** Totally, 862 blood samples were collected from children up to 12 years old from nomadic tribes of the studied area. Before sampling, a questionnaire was filled out for each case. All the collected blood samples were examined after the plasma separating by direct agglutination test for detection of anti-*Leishmania infantum* antibodies. The cut-off titer of  $\geq 1:3200$  with specific clinical features was considered as VL.

**Results:** Altogether, 25 (2.6%) of the collected plasma samples showed anti-*Leishmania* antibodies at titers  $\geq 1:800$  and 6 of them (0.6%) showed titers  $\geq 1:3200$  with mild clinical manifestations. None of the seropositive cases had a history of kala-azar. Children of 5-8 years old showed the highest seroprevalence rate (4.1%). Also, there were not any significant differences between the rate of seropositivity in males (0.58%) and females (0.67%), ( $P = 0.225$ ).

**Conclusion:** Although the seroprevalence of VL is relatively low in children up to 12 years old from nomadic tribes of the studied area, due to the importance of the disease, the surveillance system should be monitored by health authorities.

### Introduction

Visceral leishmaniasis (VL) popularly known in humans as kala-azar caused by the species of

*Leishmania donovani* complex is a systemic parasitic disease and transmitted by the bite of female sandflies. *Canis familiaris* (domestic dogs) are main VL reservoir hosts in Mediterranean type of VL (1). VL is the most severe form of leishmaniasis in the world, which is responsible about 500,000 new cases each year and about 59,000 deaths annually (2). Approximately, 100-300 new cases of VL

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reported in Iran annually (3). The parasite migrates to the mononuclear phagocyte system in the liver, spleen, and bone marrow and if left untreated in most cases can cause death (4, 5). Kala-azar is characterized by fever, weight loss, hepatosplenomegaly, anemia, and reduction in various types of peripheral blood cells. It has a high mortality rate in untreated patients, malnourished children and in cases of VL/HIV co-infection (6). Diagnosis of VL is accomplished by parasitological methods (demonstration of amastigotes in biopsies of aspirates or by culture of parasite), serological tests, and molecular methods. Parasitological and molecular methods are painful and require expensive lab facilities but serological tests, specifically direct agglutination test (DAT), do not require expensive lab equipment in comparison to other methods while are very sensitive. So they are suitable for use under field conditions (7). In this study, DAT was used as serodiagnostic tool because it is simple, cost-effective, and sensitive/specific valid test (sensitivity and specificity of the DAT were estimated 92-100% and 72-100%, respectively) (1, 2). The first case of VL in Iran was reported from the Mazandaran Province in 1949 (5). At least six endemic foci of VL have been known in some areas of Ardabil, East Azerbaijan, Fars, Bushehr, and recently from

Qom and northern Khorasan Provinces, Iran. Also, VL has been reported sporadically in other provinces of Iran (1, 8). In recent decades, more than 100 cases of VL have been reported in Kerman Province from southeastern of Iran. About one-third of the cases were registered from the nomadic tribes of southern Kerman Province, Iran (nomadic tribes of city of Jiroft and Anbarabad) (9, 10). The present study was conducted to determine the seroprevalence of VL in the nomadic tribes of Kerman Province for notified of present statues of VL in this area and selection and implementation of optimal control program.

## Methods

### Study area

Jiroft district with 5000 nomadic people is located in the south of Kerman Province, south-east of Iran (Figure 1). The study area has warm weather in the summer, while mild in the winter with an average altitude of 1100 m above the sea level and 13,799 km<sup>2</sup>, with four urban centers, 1228 villages, and 277,748 population. About 5000 (1.8%) nomadic people are living in this area. This investigation was carried out in the nomadic tribes of Jiroft district in a period of 12 months from February 2014 to January 2015.



Figure 1. Situation of Kerman Province in Iran and location of study areas in Kerman Province

### **Blood sampling**

This cross-sectional study was carried out as descriptive during February 2014-January 2015 for a period of 1-year. A randomized sampling method was used for the sample collection. A questionnaire was completed for each individual, recording demographic characteristics (age, gender, location, symptoms, history of VL, and contact with dog). Blood samples were collected from 862 children up to 12 years old and 96 persons of adults [511 (53.3%) samples from males and 447 (46.7%) samples from females] in tubes containing heparin as anticoagulant and processed them 4-10 h after collection. Plasma samples were separated by centrifugation and stored at  $-20^{\circ}\text{C}$  for subsequent serologic studies.

### **Preparation of DAT antigen**

The *Leishmania infantum* antigens for this survey were prepared in the Protozoology Unit of the School of Public Health in the Tehran University of Medical Sciences. The principal phases of the procedure for preparing DAT antigen were mass production of promastigotes of Iranian strain of *L. infantum* [MCAN/IR/07/Moheb-gh. (GenBank accession no. FJ555210)] in RPMI1640 medium (Biosera, South America) plus 10% fetal bovine serum (Biosera, South America), following tripsinization of the parasites, staining with Coomassie brilliant blue R-250 (Sigma, USA) and fixing with formaldehyde 1.2% (8, 11, 12).

### **DAT**

All the collected plasma samples were examined by DAT. For titration of *Leishmania*-specific antibodies followed the general procedures described by Mohebbali et al. (8). At the first, for screening purposes, two dilutions of 1:800 and 1:3200 were made and tested. The samples that were positive with titer 1:800 were diluted up to 1:102400 in a V-shaped microtiter plate into a dilution fluid containing 0.9% saline and 0.78% 2-mercaptoethanol. One equal volume (50  $\mu\text{l}$ ) of antigen suspension was added to each well. The results were read after 18-24 h incubation in a wet room at room temperature. The highest dilution at which agglutination was still visible in comparison with positive and negative controls

titer was defined as the titer of sample. Compact blue dots were scored as negative and large diffuse blue mats as positive. The seropositive cases were introduced to the Health and Medical Center of Jiroft district to received appropriate treatment if necessary. Antigen control well (antigen and diluent plasma only) and known negative and positive controls were tested in each plate daily. Titers of  $\geq 1:3200$  were considered as seropositive (8, 13-17); the cut-off was based on previous studies (13, 16, 18).

### **Statistical analysis**

Chi-squared and Fisher exact tests were used to compare seroprevalence values relative to gender and age. Analyses were performed with SPSS (version 13.5; SPSS Inc., Chicago, IL, USA) with  $P < 0.050$  and the value was considered as statistically significant.

### **Results**

Totally, 958 blood samples collected from the nomadic tribes of south of Kerman Province, 862 (90%) were collected from children up to 12 years old, and 96 samples (10%) were from adults. Totally, 511 (53.3%) of 958 of the studied population were male, and 447 (46.7%) were female. Totally, 25 (2.6%) of the plasma samples showed anti-*Leishmania* antibodies at titers  $\geq 1:800$  and from these only 6 cases (0.6%) showed anti-*Leishmania* antibodies at titers  $\geq 1:3200$  (Table 1) with mild fever, weakness, and paleness. No statistically significant differences between human *Leishmania* infection ( $\geq 1:3200$ ) and gender were observed ( $P = 0.225$ ). Frequency of anti-*Leishmania* antibody titers with DAT according to the age groups is shown in table 2. The highest percentage of seropositivity was seen in age groups of 5-8 years old (1.3%).

Age and gender distribution of samples is shown in table 3. About 53.3% of samples were collected from males and 46.7% from females. The most and the least samples were collected from age groups of 5-8 years old (33.1%) and adults  $> 12$  years old (10%), respectively. Table 4 shows titers of anti-*L. infantum* antibodies in six seropositive cases according to the age, gender and tribe in the south of Kerman Province.

**Table 1.** Seroprevalence of human visceral *L. infantum* infection by direct agglutination test with anti-*L. infantum* antibodies by gender in nomadic tribes of south of Kerman Province, 2014

Gender	Number of examined	Antibody Titer						Total	
		1:800		1:1600		≥ 1:3200		Number of examined	%
		Number of examined	%	Number of examined	%	Number of examined	%		
Male	511	9	1.8	2	0.4	3	0.6	14	2.7
Female	447	3	0.7	5	1.1	3	0.7	11	2.5
Total	958	12	1.3	7	0.7	6	0.6	25	2.6

*L. infantum: Leishmania infantum*

**Table 2.** Seroprevalence of human visceral *Leishmania infantum* infection by direct agglutination test with anti-*Leishmania infantum* antibodies by age group in nomadic tribes of south of Kerman Province, 2014

Age group (years)	Number of examined	Antibody Titer						Total	
		1:800		1:1600		≥ 1:3200		Number of examined	%
		Number of examined	%	Number of examined	%	Number of examined	%		
<0-4	290	3	1	1	0.3	1	0.3	5	1.7
5-8	317	6	1.9	3	0.9	4	1.3	13	4.1
9-12	255	3	1.2	3	1.2	1	0.4	7	2.7
>12	96	0	0	0	0	0	0	0	0
Total	958	12	1.3	7	0.7	6	0.6	25	2.6

*L. infantum: Leishmania infantum*

**Table 3.** Distribution of studied population for detection of seroprevalence of human visceral *L. infantum* infection by gender and age groups in nomadic tribes of south of Kerman Province, 2014

Age group (years)	Male		Female		Total	
	Number of examined	%	Number of examined	%	Number of examined	%
< 0-4	164	56.6	126	43.4	290	30.3
5-8	179	56.5	138	43.5	317	33.1
9-12	148	58	107	42	255	26.6
> 12	20	20.8	76	79.2	96	10
Total	511	53.3	447	46.7	958	100

*L. infantum: Leishmania infantum*

**Table 4.** Anti-*L. infantum* antibody titers of six seropositive cases of visceral *L. infantum* infection by direct agglutination test with respect to their age, gender, and locality in nomadic tribes of south of Kerman Province, 2014

Case No.	Age (years)	Gender	Location	Tribe	Antibody Titer
1	5	Male	Jiroft	Sargaz	1:6400
2	8	Female	Jiroft	Sargaz	1:3200
3	4	Male	Jiroft	Abshoor	1:3200
4	7	Female	Anbarabad	Tale shiraz	1:3200
5	10	Male	Anbarabad	Haji abad	1:3200
6	5	Female	Anbarabad	Haji abad	1:3200

## Discussion

Mediterranean type of VL caused by *L. infantum* is endemic in some parts of Iran such as northwestern and southern regions (8, 18-21), and sporadic form of the disease occurred in other parts of the country (8). In recent decades, more than 100 cases of VL have been reported in Kerman Province approximately one-third of the cases were registered from the nomadic tribes of southern Kerman Province (nomadic tribes of city of Jiroft and Anbarabad) (9, 10).

Due to the rural and nomadic lifestyle of the peoples of southern Kerman Province and interesting to keep dogs as pet or sheepdog, they are considered as high-risk peoples. The tribes' people in south of Kerman province (Soleimani and Jebalbarezis tribes) live in sheds and have more environmental exposure than others. These people travel with their flocks each year from the summer highland quarters in Baft district to winter quarters, on lower (and warmer) lands in Jiroft district of south of Kerman. It seems that tribes' dogs are as main reservoirs for the

disease in the nomadic tribes of south of Kerman. Since no serological study had been performed in last decade in south of Kerman Province, this study was conducted to evaluate the epidemiological aspects of VL in this area. DAT was used in this study for detection of *L. infantum* infection because the results of the DAT for detection of *L. infantum* infection in humans were excellent and its highly specificity and sensitivity (8). In 2006, Asgari et al. for determination of the VL prevalence in Qashqaei tribes in south of Fars Province, collected 321 serum samples from up to 12 years old children and tested them by DAT. They found 1.86% of seropositivity in Qashqaei tribes (22).

A serological survey was carried out by Mohebbali et al. During 2002-2005 in some parts of the country that results were as follows: Ardabil Province [Meshkinshahr (3.6%), Germe (2.8%), Ardabil, Pars-Abad and Khalkhal (5.1%)], Chahar Mahal and Bakhtiari Province [Koohrang (2.3%)], Fars Province [Mamasani (1.9%)], Lorestan Province [Poshtkuh (1.3%)], Kohgiluyeh and Bouir Ahmad Province [Yasuj (1.5%)], and Khorasan Province [Bojnurd and Shirvan, (0.46%)] (8). In Qom Province, Fakhar et al. found 1.7% of seropositivity in eight villages of Ghahan that three of seven seropositive cases had a previous history of VL and majority of the cases were among children of 7-10 years of age (23). In our study, there were not any seropositive cases with a history of kala-azar. Mohebbali et al. found 3.4% of seropositivity in Dashti and Dashtestan districts of Bushehr Province (24). A serological investigation was carried out by Mahmoudvand et al. in Baft district, Kerman Province, in this survey from 1476 collected human serum samples, 23 cases (1.55%) showed anti-*Leishmania* antibodies at titers of 1:800 and 1:1600, and 14 cases (0.95%) showed anti-*L. infantum* antibodies at titers of  $\geq$  1:3200. According to the mentioned study, VL is endemic in southwestern regions of Kerman Province; according to our study, VL is a sporadic disease in south of Kerman Province. Also, in our study, no significant difference between male and female was seen (0.58% of the seropositive cases were male and 0.67%

female), similar results were found by Mahmoudvand et al. in 2011 (5). In some of previous studies conducted in Iran, there has been no agreement in consistency of the data between males and females (5, 8, 23). These differences seem to be associated with method of sampling, number of population, and the presence or absence of clinical symptoms.

## Conclusion

It seems that kala-azar in the nomadic tribes of south of Kerman Province is a sporadic disease. This kind of information is helpful in selection and implementation of the optimal control program. We suggest that for prevention of VL in this area, nomadic tribes must be settled in village homes instead of shed where they have more exposure to environmental risk factors and fewer facilities. Also, the treatment of dogs as reservoir, screening high-risk peoples and ownership dogs by periodic DAT and treatment of infected cases can be effective. The study on vectors and probable reservoirs are recommended.

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