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**Original Article**

# Serological Survey and Associated Risk Factors of Visceral Leishmaniasis in Qom Province, Central Iran

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**Abstract**

**Background:** Visceral leishmaniasis (VL) or kala-azar is considered as a parasitic disease caused by the species of *Leishmania donovani* complex which is intracellular parasites. This systemic disease is endemic in some parts of provinces of Iran. The aim of this study was to determine the seroprevalence of VL in Qom Province, central Iran using direct agglutination test (DAT).

**Methods:** Overall, 1564 serum samples (800 males and 764 females) were collected from selected subjects by randomized cluster sampling in 2011-2012. Sera were tested and analyzed by DAT. Before sampling; a questionnaire was filled out for each case. Data were analyzed using Chi-square test and multivariate logistic regression for risk factors analysis.

**Results:** Of 1564 individuals, 53 cases (3.38%) showed *Leishmania* specific antibodies as follows: with 1:400 titer 16 cases (1.02%), with 1:800 titer 20 cases (1.27%), with 1:1600 titer 16 cases (1.02%) whereas only one subject (0.06%) showed titers of ≥ 1:3200. There was no significant association between VL seropositivity and gender, age group and occupation. Binary logistic regression showed that rural areas was 0.44 times at higher risk of infection than urban areas (OR= 0.44; %95 CI= 0.25- 0.78).

**Conclusion:** Although the seroprevalence of VL is relatively low in Qom Province, yet due to the importance of the disease, the surveillance system should be monitored by health authorities.

**Keywords:** Visceral Leishmaniasis, Seroepidemiology, Prevalence, Direct agglutination test, Human, Iran

## Introduction

Visceral leishmaniasis (VL) or Dumdum fever is a vector-borne disease caused by the *Leishmania infantum donovani* complex. VL is a systemic disease considered as the most devastating type of leishmaniasis, since it usually causes death in untreated cases and many cases of deaths are left unrecognized (1). Even in cases of treatment, it may result in case-fatality rates of 10–20%. It is estimated that about 500,000 episodes and 59,000 deaths occur annually owing to this type of leishmaniasis (2). VL is the second-largest parasitic killer in the world following malaria (3).

The clinical features of VL include fever, weight loss, fatigue, mucosal ulcers, anemia, and substantial hyperplasia of the liver and spleen that these symptoms can be easily mistaken with other febrile disease (4, 5). Of particular concern, based on the World Health Organization (WHO), is the

emerging problem of HIV/VL co-infection and 34 countries reporting *Leishmania* / HIV co-infection worldwide (6). VL is a cosmopolitan disease and distributed in the Middle East region. In addition, domestic dogs (*Canis familiaris*) are known as major reservoir hosts of VL (4).

Mediterranean VL is considered endemic in some areas of Iran including Ardabil (Moghan and Meshkin shahr), eastern Azerbaijan (Ahar and Kalibar), Bushehr (Dashti and Dashtestan) Fars (Darab, Firouz abad Noor abad and Jahrom), northern Khorasan and Qom (Khaljestan) districts. It has been reported sporadically in other provinces of the country (7-12).

The aim of this study was to determine the seroprevalence and risk factors association of VL in Qom Province, central Iran using direct agglutination test (DAT).

## Materials and Methods

### *Study area*

Qom Province located on the south of Tehran and central Iran is of 11238 sq. km. The province is connected to the Semnan Province in the east, to the Isfahan Province in the south and to the Markazi Province from south-west to north-west (Fig.1). The population is estimated as one million. The province comprises of 1 city, 5 towns, 4 districts, and 936 habitations out of which 356 are populated (Source: http://amar.sci.org.ir/index_e.aspx).

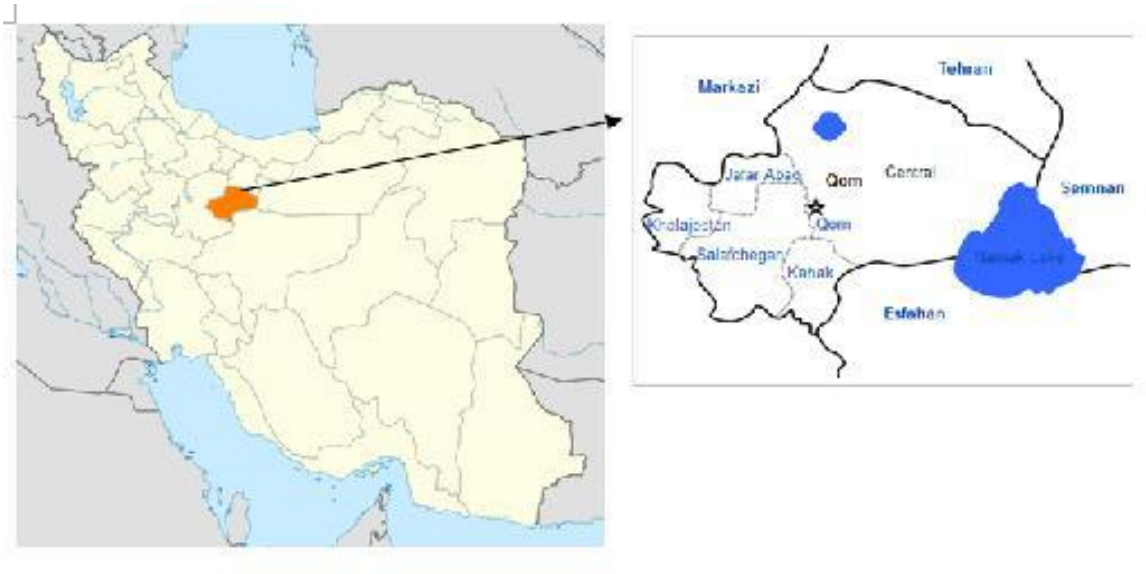


**Fig. 1:** Geographical locations of Qom Province in Iran where our serum samples were collected.

### *Blood sampling*

A cross-sectional study was performed on urban and rural populations of Qom Province in 2011-2012. Household data were obtained from local health authorities and one random person of one family out of twenty seven families was selected. Totally, 1564 serum samples (800 males and 764 females) were collected from 700 urban and 864 rural areas by randomized cluster sampling (Table1 and 2).

**Table 1:** Study areas and number of collected serum for detection of seroprevalence of human visceral Leishmania infection in Qom Province

| Area | No. of sera |
|---|---|
| Urban | 700 |
| Kahak | 264 |
| Ghamrod & Ghanavat | 163 |
| Ghaleecham & Salafcheghan | 154 |
| Ghahan & Dastjerd | 151 |
| Jaafarieh | 150 |
| Total | 1564 |

An informed consent document was taken from every participant. A questionnaire was filled out for each individual to obtain information then the blood sample was taken from each participant and transferred to sera separated the laboratory of the Amiralmomenin Polyclinic, Qom, Iran.

Sera were sent to Leishmaniasis Laboratory, Dept. of Medical Parasitology, School of Public Health, Tehran University of Medical Sciences, Iran for examination with DAT.

**Table 2:** Distribution of studied population for detection of seroprevalence of human visceral *Leishmania infantum* infection by gender, residual area, occupation, dog keeping and education in Qom Province, central Iran

| Sex | | Location | | Occupation | | | | Dog keeping | | Education | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Male | female | Urban | Rural | Farmer | Householder | Schooling | Other | Yes | No | Educated | Illiterate | |
| 800 | 764 | 700 | 864 | 201 | 571 | 166 | 628 | 184 | 1380 | 865 | 699 | 1564 |

### *Direct agglutination test (DAT)*

DAT antigen was made in the Protozoalogy Unit of the School of Public Health, Tehran University of Medical Sciences. The principal phases of the procedure for preparing DAT antigen were mass production of promastigotes of *Leishmania infantum* (MCAN/IR/07/Moheb-gh.), (GenBank accession (no. FJ555210) (Iranian strain) in RPMI1640 plus 10% fetal bovine serum, tripsinization of the parasites, fixing with formaldehyde 2% and staining with Coomassie brilliant blue. The human sera were tested by DAT, initially, for screening purposes; dilutions were made at 1:400 and 1:3200 for human's samples.

Negative control wells (antigen only) and known negative and positive controls were tested in each plate daily. The positive standard control serum was prepared from VL patients with *L. infantum* infection from the endemic areas. The cut off titer was determined as 1:3200, specific *Leishmania* antibodies at a titer of 1:3200 and upper were considered as positive (13, 14).

### *Statistical analysis*

The data were analyzed using SPSS 16 program. Odds ratios for risk factors analysis were calculated by multivariate logistic regression model. $P<0.05$ was considered as significant.

## Results

Of 1564 individuals, 53 cases (3.38%) showed *Leishmania* specific antibodies as follows: with 1:400 titer 16 cases (1.02%), with 1:800 titer 20 cases (1.27%), with 1:1600 titer 16 cases (1.02%) whereas only one subject (0.06%) showed titers of ≥ 1:3200 (Table 3 and 4). Therefore considering the cut off titer, only one sample was regarded as positive case which belongs to a 30 years- old educated man who resides in Kahak and with no history of keeping dog.

**Table 3:** Frequency of anti-*Leishmania* antibodies titers using DAT by residual area in Qom Province, central Iran

| Residual area | 1:400 | (%) | 1:800 | (%) | 1:1600 | (%) | 1:3200 | (%) | Total | (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| Urban | 4 | 0.25 | 15 | 0.96 | 15 | 0.96 | 0 | 0 | 34 | 2.17 |
| Rural | 12 | 0.77 | 5 | 0.31 | 1 | 0.06 | 1 | 0.06 | 19 | 1.21 |
| Total | 16 | 1.02 | 20 | 1.28 | 16 | 1.02 | 1 | 0.06 | 53 | 3.38 |

Furthermore, in urban areas 34 subjects (2.17%) showed a titers of ≥ 1:400 while in rural areas were 18 cases including Kahak one sample (0.4%), Ghahan & Dastjerd 11 samples (7.3%), Ghamrod

& Ghanavat 5 samples (3.1%) and Jaafarieh one sample (0.7%). There was no significant association between VL seropositivity and gender, age group and occupation. Binary logistic regression showed that rural areas was 0.44 times at higher risk of infection than urban areas (OR= 0.44; %95 CI= 0.25- 0.78).

**Table 4:** Seroprevalence of human visceral Leishmania infection by direct agglutination test (DAT ≥ 1:3200) with anti-*Leishmania infantum* antibodies by gender in Qom Province

| Gender | No. examined | Anti-Leishmania antibody titers | | | | Total |
|---|---|---|---|---|---|---|
| | | 1:400 | 1:800 | 1:1600 | ≥1:3200 | |
| | | No. Prevalence (%) | No. Prevalence (%) | No. Prevalence (%) | No. Prevalence (%) | No .Prevalence (%) |
| Male | 800 | 10<br>0.63 | 12<br>0.76 | 10<br>0.63 | 1<br>0.06 | 33<br>2.1 |
| Female | 764 | 6<br>0.38 | 8<br>0.51 | 6<br>0.38 | 0<br>0 | 20<br>1.27 |
| Total | 1564 | 16<br>1.01 | 20<br>1.28 | 16<br>1.01 | 1<br>0.06 | 53<br>3.38 |

## Discussion

In the current study from 1564 collected human serum samples, 1 sample showed anti-*Leishmania* antibodies at titers of ≥ 1:3200 whereas 52 samples (3.3%) revealed anti-*Leishmania infantum* antibodies at titers of ≥ 1:400. Meanwhile, 16 (2.1%) subjects revealed titer of 1:1600 which is considered as suspicious cases. In this study, males showed more anti-*Leishmania* specific antibodies compared to females that is in consistent with other studies (15, 16).

This survey showed that one case in rural areas had anti-*Leishmania* antibodies at titer 1:3200 in comparison with urban areas (0 case) (*P*= 0.007). Besides, rural areas was 0.44 times at higher risk to be infected than urban areas (OR= 0.44; %95 CI= 0.25- 0.78) and multivariate analysis showed that rural areas was 0.45 times at higher risk of infection than urban areas (OR= 0.45; %95 CI=0.23-0.89). This fact may be associated hygienic level in rural areas. There was no significant association between VL seropositivity, sex and age group.

According to literature review the seroprevalence of VL using DAT at titers ≥ 1:3200 in other parts of the Iran was as follows: Mazandaran Province 0.% (17), Kermanshah Province 0.33% (18), Khorasan Province [Bojnurd and Shirvan, 0.46%] (13),Kerman Province [Baft 0.95%] (19), Chahar Mahal & Bakhtiari province [Poshtkuh 1.3%] (20), Kohgiloyeh & Bouir ahmad Province 3.1% (21), Bushehr Province [Dashti and Dashtestan 3.4%] (10), Ardabil Province [Germi 2.8%, Meshkinshahr 6.3%, Pars- Abad and Khalkhal 5.1%] (7).

In addition, a similar investigation was carried out on 416 human sera samples in 8 villages of Ghahan, Qom Province using DAT for detection of *Leishmania* antibodies. Totally, 7 cases (1.7%) were positive with titers 1:3200 and above that three of seropositive cases had a previous history of VL (12). Our findings indicated that the rate of seropositivity in Qom Province is fewer than all above mentioned areas except Mazandaran Province.

In the present study, DAT was employed to detect *Leishmania* antibodies for a couple of reasons. Two main features of serological methods, being highly sensitive and non-invasive, make them appropriate for use in field conditions (13). The various serological methods including ELISA, DAT and IFAT (indirect fluorescent antibody test) are available for diagnosis of VL. Due to simplicity, validity, economical benefits, high sensitivity and specificity, DAT was used in several epidemiologic studies in Iran with large-scale screening of human. The sensitivity and specificity of this assay

at cut-off titer 1:3200 varies between 90–100% and 72–100% respectively (22, 23).

## Conclusion

The present study showed that the risk of VL is still remained in this region. To control VL in this area, further investigation on sand flies fauna and canines as reservoir hosts of VL are highly recommended, and also treatment of suspected human cases should be considered. Continuous serological researches and preventive measures should be taken into consideration owing to the significance of the disease.

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