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ORIGINAL ARTICLE

Seroepidemiological study on visceral leishmaniasis in an endemic focus of central Iran during 2017

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Abstract Visceral leishmaniasis (VL) is a serious parasitic disease that occurs in some areas of Iran as endemic and sporadic forms. Qom province in central Iran is one of the endemic foci of VL, which is an important issue due to existence of local population density, foreign travelers and resided migrants. This descriptive cross-sectional study that was conducted with the aim of evaluating seroprevalence of VL in the studied area. The research considers various risk factors like immigration and tourism for planning prevention and disease control programs. Random cluster sample selection applied and 960 blood samples collected from children up to 12 years of age. The samples were taken from 22 villages in four clusters, during 2017. A questionnaire was given to each individual after obtaining parental consent. All collected sera assessed by Direct Agglutination Test to detect anti-*Leishmania infantum* antibodies. The antibody titers of $\geq$ 1:3200, accompanied by clinical symptoms, considered as VL disease. Eventually, 3 (0.3%) cases showed anti-*L. infantum* antibodies with titers of $\geq$ 1:1600, while 2 of them (0.2%) had antibody titers of 1:3200 with no clinical manifestations. In order to investigate the increase of antibody, resampling performed after 2–3 weeks in which no antibody rising observed. The findings indicate that distribution pattern of VL is changing from endemic to sporadic form in rural areas of Qom province. Therefore, it is necessary to continue the surveillance by public health centers besides conducting further studies on VL reservoirs and vectors with the aim of VL control in the area.

Keywords *Leishmania infantum* · Visceral leishmaniasis · Seroepidemiological · DAT · Iran

Introduction

Visceral leishmaniasis (VL), also known as Kala-azar, is a serious zoonotic parasitic infectious disease transmitted by bites of some species of infected female sand flies to humans and sensitive animal hosts. The main causative agent of the disease is Leishmania species involving reticuloendothelial system macrophages leading to fever, anemia and hepatosplenomegaly in human. Epidemiologically, VL occurs as endemic, epidemic and sporadic forms with near 95% mortality rate within 2 years, if left untreated (Mohebali 2013). VL is endemic in more than 56 countries with over 50,000–90,000 new cases annually, including Iran (WHO 2018). Since the first report of VL in Iran that was released 70 years ago, the number of VL cases has been increasing with the development of serological diagnostic methods.

The main cause of Mediterranean VL in Iran is *Leishmania infantum*, isolated from various species of *phlebotomus* sand flies. Major reservoirs of the parasite are domestic and stray dogs. Based on epidemiological studies carried out in Iran, VL has endemic foci in some areas of the country and the population at risk for the disease are children up to 12 years old, especially in ages 1–5 years, residing in rural areas (Mohebali et al. 2011). In Qom province, located in central Iran, the first introduction of VL as an endemic focus was recorded in 2001 with

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prevalence rate of 1.7% but there was no report of the disease after that (Fakhar et al. 2004). New VL cases reported over the last 5 years in Qom province, seemed to be important due to the increased immigrants and tourists travelling to the area and health policy. Concerning the lack of comprehensive epidemiological studies to evaluate the status of the disease, this study was conducted with the aim of determining Kala-azar seroprevalence among rural populations of Qom province for diagnosis, treatment and program planning.

Materials and methods

This cross-sectional descriptive study was carried out in 2017 to investigate the seroepidemiology aspects of visceral leishmaniasis among children up to 12 years old in endemic rural areas of the study area. Qom Province, one of the 31 provinces in Iran, is located near the central desert with 11,526 km^2 area, covering 0.89% of the total country. The province population was near 1,292,000 in 2016 with more than 60,000 people residing in rural areas. The province contains a city, five counties, nine rural districts, and 256 villages (Fig. 1). The climate varies between desert and semi-desert to temperate. The highest rainfall of the province recorded in high altitude regions covering 25% of the province area, including two counties: Kahak and Dastjerd located in the south and west parts of the province. Qom province is one of immigrant provinces in the country with near 120,000 migrants, more than 10% of total province's population, in 2016 (IMO 2015; PHCO 2016). Placement of Qom city along the communication routes beside tourist attraction due to religious importance, brings many Iranian and foreign travelers to the area that increases the importance of vector borne diseases control (Fig. 1).

According to several epidemiological studies conducted in Iran, the population at risk for VL are the children up to 12 years of age in rural and nomadic areas (Mohebali 2013). Hence, the sampling was carried out on children residing in the province villages in 2017. Additionally, in order to evaluate the history of parasite exposing, 10% of the sample size allocated to residents over 12 years old. By using Cochran's Method, the sample size is estimated to be 904. Random cluster sample selection is applied in four clusters (corresponding to four regions: Kahak, Fordo, Dastjerd and Ghahan). Overall, 960 samples were collected from randomly selected children living in 22 villages, located in two districts of Qom province namely Khalajestan and Kahak districts. Before sampling, an informed consent letter was signed by each individual in cooperation with the Rural Health Care Centers, observing the ethics while a checklist was used to record epidemiologic information. Residence in the study area was the main entrance criterion consisting those who had a temporary summer residence, considering presence of part of the disease commune period in the summer. Dissatisfaction of any child's parents and dissociation of children from sampling was respected as withdrawal criterion.

Near 100–150 µl blood samples were collected from each person using disposable sterile lancet and non-heparinized capillary tubes. The collected samples were transferred in cold chain condition to prevent the antibody being destroyed and hemolyzed until serum separation in

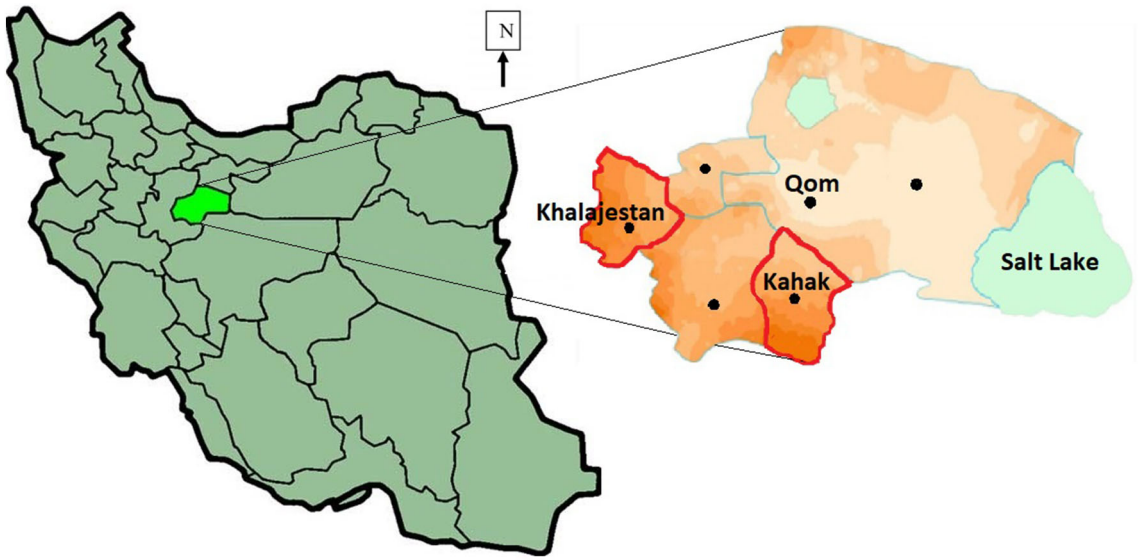

Fig. 1 Location of Qom province in Iran and topographic map of selected districts

the laboratory by using micro-centrifuge in 1000*g* for 3–5 min. All sera were stored in − 20 °C until DAT application. DAT method is designed based on agglutination of promastigote forms of *L. infantum* enclosing to diluted serum sample in presence of anti-Leishmania antibodies. Preparation of the antigen used in this study was performed in Leishmaniasis laboratory of Parasitology Department, School of Public Health, Tehran University of Medical Sciences, Iran. The process of antigen preparation included several steps starting by mass production of promastigotes of the Iranian strain of *L. infantum* [MCAN/IR/07/Moheb-gh. (Gen Bank accession no. FJ555210)] in RPMI-1640 medium (Biosera, South America) containing 10% of fetal calf serum (Biosera, South America), following trypsinization of the parasites and staining with coomassie brilliant blue R-250 (Sigma, St. Louis, Missouri, USA) and eventually fixation in 1.2% formaldehyde (Mohebali et al. 2006). Serial dilution of the serum samples prepared with a solution of 0.9% saline and 0.78% 2-mercaptoethanol at the ratio of 1:10–1:102400 in V-Bottom polystyrene Microplates. Afterwards, 50 µl of DAT-antigen suspension added to each well. To ensure the accuracy of the tests, positive and negative control sera were used with each test series. The plates were checked after 12–18 h of incubation in a wet chamber at room temperature by naked eye on a white background sheet in sufficient light, the highest dilution with a visible agglutination considered as the antibody titer, in comparison with the positive and negative controls. Negative result was like a compact blue spot at the bottom of the well and agglutination seemed like a matt cloud, spread in the well. In the screening stage, only the dilution of 1:800 was examined to detect suspected samples. Then the antibody titration performed on the samples with positive result in the screening stage. The cut-off titer considered as 1:3200, reported as Leishmania infection, accompanying with clinical manifestations, obtained from numerous earlier studies (Mohebali et al. 2006, 2015). In order to investigate the antibody increase and also ensuring the results, re-sampling was carried out within 2–3 weeks after the first sampling and the antibody titration test was performed over again. The seropositive cases were referred to pediatric specialist for further clinical examinations and received appropriate treatment, if needed.

All collected data was analyzed using IBM SPSS Statistics. Version 22. Fisher's exact and Chi square tests, with 95% confidence interval, were preformed to compare positive values relative to age, gender and nationality. Existence of statistical significance was assumed if P value ≤ 0.05.

Results

From a total of 960 serum samples collected from the studied area, including 494 males and 466 females, with 1.06 male/female ratio, 7 cases (0.7%) showed an antibody titer of ± 1:800 in the screening stage, considered as suspicious. In result of titration stage, three cases (3%) with the antibody titers of 1:3200 and one case (1.0%) with 1:1600 antibody titer identified in need of follow up. After resampling and Direct Agglutination Test (DAT) applied to suspicious cases, no antibody rising identified in any case, but two cases with anti-*Leishmania* antibody titer of 1:3200 and one case who showed an antibody titer of 1:1600 were referred to pediatrician in order to check up for VL clinical symptoms and further treatment if needed. One of the seropositive children, a 6-years-old girl from Khaveh village in Fordow section, had a previous history of VL at the age of two, successfully treated. Another seropositive child, who was an 11-years-old Afghan boy residing in Ghahan village, did not have certain clinical manifestations, including recent persistent fever, anemia and splenomegaly, but had signs of thinness and malnutrition.

As a final point, of 960 cases examined in this study, 3 (0.3%) cases were seropositive, so that 2 (0.2%) of them showed anti-*Leishmania* antibody titer of 1:3200, considered as asymptomatic VL infection. One of them had previous history of Kala-azar; the other case (0.1%), with antibody titer of 1:1600, was seropositive that could be a sign of being exposed to *L. infantum* parasite. Descriptive statistical data of the studied population in this survey are shown in Table 1. Detailed characteristics of 3 seropositive cases are given in Table 2.

Discussion

The present study evaluates serological prevalence of visceral leishmaniasis in rural areas of Qom province, central Iran, during the year 2016. The findings indicated that 0.3% of studied population had anti-*Leishmania* antibody infection, in which 0.2% showed 1:3200 titer while one of them had previous history of Kala-azar. The first report of VL in Qom province was conducted by Fakhar in 2001 with the incidence rate of 1.7% in human and 25% in domestic dogs, led to program planning for VL control in the area (Fakhar et al. 2004). A few years later, in the results of a study on associated risk factors of VL in Qom province, sero-prevalence of VL was evaluated as 1.27% with the titer of 1:800 among 1654 human serum samples. The study did not observe significant correlation between VL seropositivity and gender or career, but rural areas were at higher risk of infection than urban areas (Rakhshanpour

Table 1 Characteristics of study population and relative seropositivity to *L. infantum* using DAT among up to 12-year old children, in rural areas of Qom province, central Iran during 2016

Characteristics	Number	Percent (%)	Positive for anti-*Leishmania* antibody	
			Number	Percent (%)
Gender				
Male	494	51.4	2	0.4
Female	466	48.6	1	0.2
Age group (years)				
< 4	309	33.2	0	0
5–8	316	32.9	2	0.6
9–12	234	23.4	1	0.4
> 12	101	10.5	0	0
Residence area				
Ghahan	274	28.4	1	0.4
Dastjerd	156	16.3	0	0
Fordo	205	21.4	1	0.5
Kahak	325	33.9	1	0.3
Parents educational level				
Illiterate	167	17.4	0	0
Elementary	571	59.5	2	0.3
High school	205	21.3	1	0.5
University	17	1–8	0	0
Nationality				
Iranian	741	77.2	1	0.1
Afghan	219	22.8	2	0.9
Parent career				
Farmer	112	11.6	1	0.9
Animal husbandry	40	4.2	0	0
Builder	71	7.4	0	0
Worker	404	42.1	2	0.5
Employee	38	4	0	0
Free jobs	295	30.7	0	0

Table 2 Characteristics of seropositive children

Case no.	Age	Gender	Residence area	Kala-azar history	Travel to VL endemic area	Nationality	Parents career	Contact with dog	Anti-Leishmania antibody titer
1	11	Male	Ghahan	No	No	Afghan	Worker	Yes	1:3200
2	7	Male	Fordo	No	No	Afghan	Worker	Yes	1:1600
3	8	Female	Kahak	Yes	No	Iranian	Farmer	Yes	1:3200

et al. 2014). Kala-azar has been a rural disease in Iran reported from nomadic tribes as well. In a survey on 6558 serum samples from different VL endemic parts of Iran from 2002 to 2005, the highest incidence of the disease reported in northeastern and northwestern Iran were 4.9% and 4.4% respectively. In the central and south-western parts of the country, VL had a lower prevalence that was between 1.5 and 1.9%, higher than the present study (Mohebali 2013). In a study in Kerman province, southern Iran, 118 VL cases with the mean age of 4.2 year were reported during 1994–2011. Seroprevalence of VL in the study ranges in different time intervals depending on samples selection, diagnostic methods and spatial distribution of the disease in southern Iran (Sharifi et al. 2017).

Likewise, as stated in an investigation conducted in rural areas of Alborz province, in north of Iran, from a total of 1007 children examined, 3.7% were positive for anti-*Leishmania* antibody (Heidari et al. 2015). Furthermore, in the studies carried out by Abbaszadeh and Ebrahimzadeh on nomadic tribes of Kerman (in the south of Iran) and Ardebil (in the north-west of Iran) provinces during 2014 and 2015, prevalence of VL was evaluated as 0.3% and 0.6%, respectively, which is proximate to the findings in the current study (Abbaszadeh-Afshar et al. 2015; Ebrahimzade-Parikhani et al. 2017).

In numerous endemic foci of Kala-azar in Iran and the world, most of VL infections remain asymptomatic. The ratio of subclinical to clinical VL cases reported in the studies in India was 10:1 and in West Bengal 8:1 (Chappuis et al. 2007; Saha et al. 2017). Elsewhere in a survey in Iraq, 20% of healthy and asymptomatic children diagnosed with anti-Leishmania antibody using DAT (Gani et al. 2010). Presence of asymptomatic infected individuals in VL endemic areas might play a role in the parasite transmission cycle. Thus, assessment of residents in VL endemic areas using serological screening tests can help the early detection of VL cases and decrease transmission rate (Topno et al. 2010). Likewise in the research aimed to evaluate sero-prevalence of VL in asymptomatic healthy children from rural areas of Fars province, the southern Iran, Anti-*Leishmania* antibodies were detected in 17 (2.8%) of 617 examined children in which the age group 5–8 year-old showed the highest sero-prevalence rate (Layegh Gigloo 2018). In studies on VL in different parts of Iran, no significant association was found between gender and serum infection, however in most studies the number of male cases was slightly higher than females, which also confirmed in this study, might had been due to males more exposure to sand fly bites (Mohebali 2013).

In the present study, two cases out of three seropositive children found to have Afghan nationality; they were included a noteworthy population of residents in the study area. In another study in rural areas of Alborz province, the central Iran, among 1007 children, 79.2% Iranian, 20.6% Afghan and 0.2% with other nationalities, 20 (54.1%) cases of Iranian and 17 (46.0%) of Afghan children showed anti-Leishmania antibody titers of 1:800. This observation indicated a significant correlation between nationality and the rate of VL seropositivity (Heidari et al. 2015). Qom province is one of the religious tourist destinations in Iran because of the existence of the holy shrine which constantly attracts many pilgrims every year. The number of tourists coming from the neighboring countries such as Iraq was increased, over recent years. Moreover, Afghan migrant workers comprise about 10% of the population in Qom province (PHCO 2016). Iraq and Afghanistan have endemic foci for VL. According to WHO reports, around 400 cases of Kala-azar occurred in Iraq in 2015 which is almost 5.5 times more than in Iran (WHO 2018). In order to investigate the origin of the infection, the date of seropositive Afghan children immigration to Iran and their last trip to Afghanistan was queried. Both boys, 7 and 11-years-old, had been living in Iran for more than 6 years, with no travel to other VL endemic areas. Moreover, due to the cross-reaction between antibodies in malaria, tuberculosis and cutaneous leishmaniasis with *L. infantum* antibodies in DAT, presence of the history of such diseases in the children was checked. Kala-azar has a 6–8 months commune period, in average. Anti-*Leishmania* antibodies lifetime in blood circulation ranges from few months to some years. Therefore, more prevalence of *L. infantum* antibody among non-Iranian children compared with Iranians (9–1%) in present study, has been random and not attributed to the history of being exposed to the parasite out of the study area (Alvar et al. 2012).

Given the major role of dogs in transmission cycle of *L. infantum* as parasite reservoirs, presence of stray or domestic dogs near the places where people live is one of VL risk factors. In a study conducted in Meshkin-Shahr city, an endemic focus of VL in the north-west of Iran, the significant population of dogs with symptomatic or asymptomatic VL, living near the residents, was recognized as a cause of high prevalence of the disease in the region. In a research on 110 domesticated dogs, 83.3% of dogs with VL symptoms and 18.6% of asymptomatic dogs were positive in DAT, and interestingly, 13.4% of the asymptomatic DAT-negative dogs were positive in parasitology exams (Moshfe et al. 2008). Therefore, regarding the role of dogs in sporadic VL cases incidence, another study on population of stray and domestic dogs in Qom province is suggested.

Conclusion

Findings of this study indicated that VL is still circulating in Qom province, the central Iran, with a low endemicity and it seems to be changing face into sporadic form. Additional epidemiological studies are required on reservoirs and vectors of the disease to plan and prevent the disease outbreak in the region.

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Compliance with ethical standards

Ethical considerations To maintain ethics, before sampling, an informed consent obtained from parents of all children participating in the study. This study had the approval code IR.TUMS.-REC.1396.2332 from the Research Ethical Review Committee of Tehran University of Medical Sciences, Tehran, Iran.

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