

Seroepidemiological study of visceral leishmaniasis in Booyerahmad district, south-west Islamic Republic of Iran

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دراسة وبائية سيرولوجية حول داء الليشمانيات الحشوي في مقاطعة بويرأحمد، في جنوب غرب جمهورية إيران الإسلامية
بهادر سرकारी، ناهيد بدرام، مهدي محب علي، عبد العلي مُشفع، محمد أمين زاركر، بهناز أخوندي، محمد رضا شيرزادي

الخلاصة: يتوطن داء الليشمانيات الحشوي في أجزاء من جمهورية إيران الإسلامية. وقد أجرى الباحثون دراسة مُستَعرضة على الأطفال في منطقة بويرأحمد في جنوب غرب البلاد. فأخذوا عينات من دم 1628 طفلاً دون سن العاشرة من مناطق مختلفة من بويرأحمد في عامي 2005 و2006، وتحروا عن أضداد الليشمانيات فعثروا عليها لدى 50 من هؤلاء الأطفال (3.1%)، عن طريق اختبار التراص المباشر (عيار الأضداد $\geq 1/3200$). ولم يكن هناك فرق يُعتدُّ به إحصائياً في الإيجابية السيرولوجية بين الجنسين (2.8% لدى الذكور و3.3% لدى الإناث). وشوهدت أعلى معدلات العدوى (5.2%) في من هُم في عمر عشر سنوات. على أن الحاجة تمسُّ لمزيد من الدراسات لاستقصاء مستودعات المرض والعوامل الناقلة له في هذه المنطقة.

ABSTRACT Visceral leishmaniasis (VL) is endemic in parts of Islamic Republic of Iran. A cross-sectional seroprevalence study was carried out in children in Booyerahmad district in the south-west of the country. Serum samples were taken from 1628 children up to 10 years old from different areas in Booyerahmad in 2005–06. Anti-*Leishmania* antibody was detected in 50 out of 1628 children (3.1%) by direct agglutination test (antibody titre $\geq 1:3200$). There was no significant difference in seropositivity between the sexes (2.8% males and 3.3% females). The highest rate of infection (5.2%) was in the age group 10 years. Further studies are needed to explore the reservoirs and vectors of the disease in this region.

Étude séro-épidémiologique sur la leishmaniose viscérale dans le district de Booyerahmad, sud-ouest de la République islamique d'Iran

RÉSUMÉ La leishmaniose viscérale est endémique dans certaines régions de la République islamique d'Iran. Une étude transversale sur la séroprévalence a été menée auprès d'enfants du district de Booyerahmad dans le sud-ouest du pays. Des échantillons de sérum ont été prélevés sur 1628 enfants âgés de 0 à 10 ans dans différentes zones de Booyerahmad en 2005-2006. L'anticorps anti-leishmania a été détecté chez 50 des 1628 enfants (3,1 %) par un test d'agglutination directe (titre des anticorps $\geq 1:3200$). Aucune différence significative entre les sexes n'a été constatée en ce qui concerne la séropositivité (2,8 % chez les garçons et 3,3 % chez les filles). Le taux d'infection le plus élevé (5,2 %) se trouvait dans le groupe des enfants de 10 ans. Des études complémentaires sont nécessaires pour étudier les réservoirs et les vecteurs de la maladie dans cette région.

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Introduction

The leishmaniasis are endemic diseases in 88 countries in the world [1]. Visceral leishmaniasis (VL) is caused by the *Leishmania donovani* complex, including *L. donovani* in the Indian subcontinent and East Africa, *L. infantum* in Mediterranean areas and the Middle East and *L. chagasi* in Latin America [2]. VL is endemic in 62 countries, 500 000 new cases occur annually and 200 million people are at risk worldwide [1].

Visceral or cutaneous forms of leishmaniasis are present in at least 20 out of the 31 provinces of the Islamic Republic of Iran and VL is endemic in the south (Fars province) and north-west (East Azarbaijan and Ardabil province) of the country [3–6]. The Mediterranean type of VL, caused by *L. infantum*, is also present in the Islamic Republic of Iran. Estimates of the seroprevalence of VL in various areas of the country range from 0.5% to 5% [7].

Cases of VL have been reported from different parts of Kohgiluyeh and Booyerahmad province in the south-west of the Islamic Republic of Iran. A review of hospital records in Yasuj, the capital of the province, revealed that during 1998–2001, 57 cases of VL were admitted to Yasuj hospitals. This means that VL is an endemic disease in this region, but the scale of the disease and seroprevalence rate are unknown. The current study was therefore conducted to determine the seroprevalence of VL in children in Booyerahmad district (the largest and most populated township of the province).

Methods

Study area and community

This epidemiological study was conducted in Booyerahmad district, south-west Islamic Republic of Iran (30°40'N, 51°35'E) in Kohgiluyeh and Booyerahmad province (Figure 1). The district covers an area of approximately

16 264 km². The region is mountainous and the vegetation of the area includes wild pistachio and tulips and most of the farmland and plains are covered with oak forests. Its population is estimated to be 697 654, among whom 49% are settled in urban areas and 51% live in rural areas. The people live mainly on animal farming (sheep, goats and cattle) and gardening.

Serum samples

Using cluster sampling 6 different areas of Booyerahmad district including the capital of the province, Yasuj, were randomly selected. The sample size was estimated based on the population of the area and a prevalence of VL of about 2% in other VL-endemic areas in the Islamic Republic of Iran. Altogether 1628 serum samples were taken from children up to 10 years old over an 11-month period of from September 2005 to July 2006. Active case finding was carried out in 6 clusters (villages) selected randomly from about 300 villages of Booyerahmad district. The

census in each selected villages was used and serum samples were taken from all children up to 10 years of age. The method for sample collection in Yasuj, which has a large population, was simple by random sampling.

Data on age, sex and place of residence were recorded in a predesigned datasheet. Blood samples were taken from the children using a lancet and microtube and each blood sample was placed on a 3 mm diameter filter paper. Finger prick blood samples (about 50 µL) were collected by trained health workers or our expert technicians. The samples were transferred to the leishmaniasis laboratory in the School of Public Health, Tehran University of Medical Sciences for analysis.

Ethical approval of the study was given by the ethics committee of Yasuj University of Medical Sciences and informed consent was obtained from the parents of participants to donate blood samples voluntarily. There were no refusals to participate as before the initiation of this project, the parents of



Figure 1 Geographical situation of Booyerahmad district, south-west Islamic Republic of Iran

Table 1 Seroprevalence of visceral leishmaniasis by sex and age in children up to 10 years old in Booyerahmad district

Vairable	No. of samples	%	DAT positive ($\geq 1:3200$)	
			No.	%
Sex				
Male	749	46.0	21	2.8
Female	879	54.0	29	3.3
Age (years)				
1-4	219	13.5	3	1.4
5	149	9.2	1	0.7
6	251	15.4	10	4.0
7	344	21.0	11	3.2
8	252	15.5	11	4.4
9	259	15.9	6	2.3
10	154	9.5	8	5.2
Total	1628	100.0	50	3.1

DAT = direct agglutination test.

children were made aware by health workers of the importance of kala azar in children

Direct agglutination test

The direct agglutination test (DAT) antigen for this study was prepared in the parasitology department of the School of Health at Tehran University of Medical Sciences. DAT was performed as described by Joshi et al. [8]. The blood spot on each filter paper was punched and serum was eluted from the paper by overnight incubation of each spot in wells with 50 μ L of distilled water. Then 10 μ L of the eluted serum was used for screening and testing for anti-*Leishmania* antibody by DAT in V-shaped

microtitre plates. An antibody titre of 1:3200 and above was considered positive [7,8].

Data analysis

Chi-squared and Fisher exact tests were used to compare seroprevalence values relative to sex, age and district. Analyses were conducted using SPSS software, version 13.5, with a *P*-value < 0.05 taken as statistically significant.

Results

From 1628 collected samples, 749 (46.0%) were from males and 879 (54.0%) from females. Anti-*Leishmania* antibody was detected in the serum of

50 of the 1628 children (3.1%) by DAT (antibody titre $\geq 1:3200$). From those 50 cases, 21 (2.8%) were males and 29 (3.3%) were females, a difference that was not statistically significant (*P* = 0.43) (Table 1).

Table 1 also shows the seroprevalence of *Leishmania* spp. infection by age in children up to 10 years age. The highest rate of infection (5.2%) was seen in children aged 10 years and the lowest rate (0.7 %) was found in the age group 5 years. However, the difference in the rate of antibody against *Leishmania* in children less than ≤ 4 year old compared with children 4+ years old was not statistically significant (*P* = 0.116).

Table 2 shows the seroprevalence of *Leishmania* infection in children in different areas of Booyerahmad district. The highest rate of infection was found in Yasuj (66.5% of samples tested positive).

Discussion

VL is a serious public health problem in many tropical and subtropical regions of the world, including the Islamic Republic of Iran. Dogs and other canines are the main reservoirs of VL in our country [9]. Serological surveys have been carried out in different parts of Islamic Republic of Iran to find out the rate of VL infection. In a comprehensive study conducted by Mohebbali et al. 12 144 serum samples were collected from 4 different geographical zones and tested by DAT [7]. It was found that the seroprevalence of human VL infection ranged from 0.46% in Khorasan to 5.1% in Ardabil province.

Cases of VL have been reported from different parts of Kohgiluyeh and Booyerahmad province in the south-west of Islamic Republic of Iran but the prevalence of the disease was previously unknown. Since the Mediterranean type of the disease is present in the Islamic Republic of Iran and the disease in this region is mostly found in infants,

Table 2 Seroprevalence of *Leishmania* infection in children in different areas of Booyerahmad district

Location	DAT positive		DAT negative		Total	
	No.	%	No.	%	No.	%
Yasuj	41	2.5	1041	63.9	1082	66.5
Dashtroom	0	0.0	49	3.0	49	3.0
Sarabtaveh	1	0.1	99	6.1	100	6.1
Sepidar	2	0.1	96	5.9	98	6.0
Madovan	3	0.2	163	10.0	166	10.2
Margoon	3	0.2	130	8.0	133	8.2
Total	50	3.1	1578	96.9	1628	100.0

DAT = direct agglutination test.

the subjects of the current study were children. The findings of our study show that anti-*Leishmania* antibody was detectable in 3.1% of the studied subjects. Comparing the results of this study with those of Mohebalı et al. shows that the prevalence of VL in Booyerahmad district is higher than most other provinces in the Islamic Republic of Iran. The way of life of the inhabitants, such as farming, keeping guard dogs and a nomadic lifestyle, might account for such a high prevalence of VL in this region [10].

The results of our study show no differences in seroprevalence of VL between boys and girls in this area. However, an association between sex and VL infection was observed in Mohebalı et al.'s study, where males had a higher rate of VL infection [7].

In conclusion, the findings of this study demonstrate that VL is an endemic disease, with a relatively high prevalence rate in Kohgiluyeh and Booyerahmad province. Further studies are needed to survey the reservoirs

and vectors of the disease in this region.

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