

# Seroepidemiological study of visceral leishmaniasis among humans and animal reservoirs in Bushehr province, Islamic Republic of Iran

M. Mohebali,<sup>1</sup> Y. Hamzavi,<sup>2</sup> G.H. Edrissian<sup>1</sup> and A. Forouzan<sup>3</sup>

دراسة سيرولوجية وبائية حول داء الليشمانيات الحشوي لدى البشر ومستودعاته الحيوانية في ولاية بوشهر في جمهورية إيران الإسلامية  
مهدي موهبالي، يزدن حمزوي، غلام حسين إدريسيان وعبد الرسول فوروزاني

خلاصة: تم إجراء دراسة لداء الليشمانيات الحشوي لدى الأطفال والبالغين في 13 قرية وقبيلة بدوية في ولاية بوشهر في جنوب إيران خلال الفترة 1995-1998، باستخدام اختبارات التراص المباشر. ومن بين عينات البلازما المأخوذة والبالغ عددها 1496 عينة، وجد أن معدل الإيجابية المصلية التي تزيد عباراتها عن 1:3200 كانت 3.4%. وكان معظم الحالات (94.1%) لدى أطفال تقل أعمارهم عن عشر سنوات. وقد شخص لدى 18 مريضاً منهم مرض الكلازار وكانت أبرز العلامات السريرية (الإكلينيكية) الحمى وضخامة الطحال. وتم كشف العدوى بالليشمانيات الطفلية لدى الكلاب وبنات آوى في المنطقة بإجراء الاختبارات الطفيلية والمصلية. وتقدم الدراسة اقتراحات لمكافحة داء الليشمانيات الحشوي بالقضاء على الحيوانات الضالة، وتشخيص الحالات بين الناس وبين الكلاب المقيمة المشتبه بإصابتها باستخدام اختبارات سيرولوجية مثل التراص المباشر، ومعالجة من تثبت إصابته.

**ABSTRACT** Using direct agglutination tests, a survey of visceral leishmaniasis was carried out among children and adults from 13 villages and from nomadic tribes in Bushehr province during 1998-99. Of the 1496 plasma samples, the overall seropositive rate (titres  $\geq$  1:3200) was 3.4%. Almost all cases (94.1%) were in children under 10 years old. Eighteen patients were diagnosed with kala azar; fever and splenomegaly were the predominant signs and symptoms. Parasitology and serology examinations of local animals identified dogs and jackals infected with *Leishmania infantum*. Suggestions for control of visceral leishmaniasis in this area are to eliminate stray dogs, identify cases among humans and suspected leashed dogs, and treat infected individuals.

## Etude séro-épidémiologique de la leishmaniose viscérale chez des réservoirs humains et animaux dans la province de Bushehr en République islamique d'Iran

**RESUME** Une enquête sur la leishmaniose viscérale utilisant des tests d'agglutination directe a été réalisée chez des enfants et des adultes de 13 villages et de tribus nomades dans la province de Bushehr en 1998-1999. Dans les 1496 échantillons de plasma, le taux global de séropositivité (titre  $\geq$  1:3200) était de 3,4 %. Presque tous les cas (94,1 %) étaient des enfants de moins de 10 ans. Chez dix-huit patients, on a diagnostiqué un kala-azar ; la fièvre et une splénomégalie constituaient les signes et symptômes prédominants. Les examens sérologiques et parasitologiques pratiqués sur les animaux locaux ont permis d'identifier les chiens et les chacals comme étant infectés par *Leishmania infantum*. Pour lutter contre la leishmaniose viscérale dans cette région, il est suggéré d'éliminer les chiens errants, d'identifier les cas chez les humains et chez les chiens en laisse suspects et de traiter les individus infectés.

<sup>1</sup>School of Public Health and Institute of Public Health Research, Teheran University of Medical Sciences, Teheran, Islamic Republic of Iran.

<sup>2</sup>Medical Faculty, Kermanshah University of Medical Sciences, Kermanshah, Islamic Republic of Iran.

<sup>3</sup>Medical Faculty, Bushehr University of Medical Sciences, Bushehr, Islamic Republic of Iran.

Received: 30/04/01; accepted: 02/08/01

## Introduction

Visceral leishmaniasis (VL) or kala azar is an endemic disease in parts of the Islamic Republic of Iran: in some areas of Fars province in the south and in Ardabile and East Azerbaijan in the north-west [1]. In Bushehr province a high number of cases of kala azar were recorded during the last decade, which indicates that VL may also be endemic in some areas of this province. From 1991 to 1997, 92 cases of kala azar were diagnosed in the Dashti and Dashtestan districts of Bushehr. These districts are near Firooz Abad, Ghir and Jahrum in the southern part of Fars province, areas that are known to be endemic foci of kala azar in the Islamic Republic of Iran [2].

In a preliminary study in 1993, *Leishmania infantum* was isolated from an infected dog from Bushehr city and characterized by random amplified polymorphic DNA, polymerase chain reaction (RAPD-PCR) testing [2]. In addition, the following species of sand-fly have been discovered in the Dashti and Dashtestan districts: *Phlebotomus (Phlebotomus) papatasi*, *P. (Larroussius) major*, *P. (Larr.) keshishiani*, *P. (Paraphlebotomus) alexandri*, *P. (Par.) sergenti*, *P. (Par.) jacusieli*, *P. (Adlerius) longiductus* and *P. (Par.) halepensis*.

This study was carried out during 1998–99 in the villages and nomadic tribes of the Dashti and Dashtestan districts of Bushehr province. The aim was to determine the seroprevalence of VL in the area and identify the animal reservoirs of the disease in order to prevent and control the infection in humans.

## Methods

The study area is situated in the southern slope of the Zagros range of mountains, in

the north and north-east of Bushehr province, at an altitude of 60 to 65 metres. The population is about 276 600. The investigation was carried out over a period of 12 months from September 1998 to October 1999 on the residents of 13 villages and the members of nomadic tribes in Dashti and Dashtestan districts. Based on the authors' previous work [2] the selected villages were thought to be likely endemic foci of VL in Bushehr province.

Plasma samples were collected in heparinized capillary tubes from 1340 children under 15 years old and 156 adults, and from 63 individuals from nomadic tribes. Also, serum samples were prepared from 36 suspected patients who had been referred to two local diagnostic laboratories for kala azar in the districts.

In order to research the animal reservoirs of VL in the study areas, samples were taken from a number of suspected animals: 105 dogs, 10 jackals, 4 foxes and 152 rodents. Blood samples were collected and some specimens from spleen, liver and bone marrow were cultured on Novy-MacNeal-Nicolle, liver infusion broth tryptose (NNN-LIT) media. Impression smears were also prepared from the spleen and liver of each animal. Spleen suspensions from these animals were inoculated intraperitoneally into golden hamsters.

One series of plasma samples collected from humans and animals was analysed in the local laboratories by the direct agglutination test (DAT) [3,4]. *L. infantum* LON49 (MCAN/IR/94/Moheb1) was used for the preparation of DA antigen. Another series of plasma samples was transferred to the Protozoology Unit at the School of Public Health, Teheran University of Medical Sciences. Titres of  $\geq 1:3200$  for human samples and  $\geq 1:320$  for animal serum specimens were considered as seropositive.

The smears prepared from spleen and liver of suspected animals were Giemsa-stained and examined carefully by light microscope at high magnification ( $\times 1000$ ) for the presence of Leishman bodies. The culture samples in NNN-LIT media were checked for promastigotes twice a week for 6 weeks. The inoculated hamsters were killed 3–6 months after inoculation, smears were prepared from their spleens and livers and examined carefully for the presence of Leishman bodies.

*Leishmania* promastigotes which had been isolated from the spleens of the dogs and one jackal after mass production in RPMI 1640 media were analysed by RAPD-PCR in the Medical Faculty of Shiraz University of Medical Sciences [5,6].

## Results

Of the 1496 plasma samples prepared from children and adults resident in the villages, 51 cases (3.4%) showed anti-*Leishmania*

antibodies in titres of  $\geq 1:3200$  by DAT (Table 1). All the cases were children under 15 years and most of them (48 cases, 94.1%) were under 10 years of age. The highest proportion of positive cases was in children 1–2 years old and the rate among children decreased with age. A number of seropositive cases (15) were found among children with no previous history of kala azar. No anti-*Leishmania* antibodies were detected in adults with titres  $\geq 1:3200$ , but 9 individuals showed DAT titres between 1:800 and 1:1600 (Table 1).

About 3.8% of the seropositive individuals were males and 3.0% females, giving a male to female ratio of 1.04:1. The geometric mean reciprocal titre (GMRT) in females (15 848.9) was much higher than in males (5214.8).

Eighteen patients were diagnosed clinically with kala azar. All of them were under 9 years old and 16 (89%) were under 4 years. All of them were seropositive with DAT analysis (titres  $\geq 1:3200$ ). Fever (89% of cases) and splenomegaly (83%) were

Table 1 Results of direct agglutination tests from 1496 plasma samples by age

Age (years)	Total	Anti- <i>Leishmania</i> antibody titres						GMRT ( $\geq 1:300$ )
		$\leq 1:800$		1:800 to 1:1600		1:3200 to 1:102 400		
		No.	%	No.	%	No.	%	
< 1	68	65	95.6	2	2.9	1	1.5	1584.9
1–2	140	127	90.7	2	1.4	11	7.9	3981.1
3–4	203	180	88.7	11	5.4	12	5.9	1995.5
5–9	656	619	94.4	13	2.0	24	3.6	1995.3
10–14	273	264	96.7	6	2.2	3	1.1	1258.9
$\geq 15$	156	154	98.7	2	1.3	0	0.0	912.0
Total	1496	1409	94.2	36	2.4	51	3.4	

GMRT = geometric mean reciprocal titre.

the most common signs and symptoms. Kala azar cases were more frequent in nomads, and most seropositive cases were found among nomadic children.

The results of immunofluorescence assay (IFA), DAT and enzyme-linked immunosorbent assay (ELISA) of suspected animals are summarized in Table 2. In parasitology examinations, amastigotes were found in the smears prepared from the spleen of three dogs in the Dashti locality, and one infected jackal that was shot in the same area. All of the four isolates were identified as *L. infantum* by RAPD-PCR. No *Leishmania* parasites were observed in 85 rodents and 4 foxes. None of the 30 golden hamsters that were inoculated by specimens from infected animals was found infected 6 months after inoculation.

## Discussion

In this study, serological surveys using DAT analysis showed that 3.4% of the population in the study areas had anti-*Leishmania* antibodies in titres of  $\geq 1:3200$ , all of them children under 15 years old. A num-

ber of seropositive cases were found among children with no previous history of kala azar. The peak number of cases was in children 1–2 years old and the seropositive rate decreased with increasing of age of the children. Prior studies in the Islamic Republic of Iran have shown a seropositive rate of about 50% in the age group 1–2 years and 96% of seropositive cases in children up to 8 years old [1–3]. No anti-*Leishmania* antibodies were detected with titres  $\geq 1:3200$  in adults.

About 3.8% of seropositive individuals were males and 3.0% females. No statistically significant difference ( $P < 0.05$ ) was observed between them. Cross-sectional IFA and DAT serological surveys of VL in endemic foci of the Islamic Republic of Iran showed that females are exposed and become infected at least as much as males. However subclinical forms of the disease may be more common in females than males. In some rural areas the rate of active kala azar cases in males may be higher than females [8]. Therefore, it does not seem that VL affects males more than females, at least in the Bushehr areas studied. Howev-

Table 2 Results of parasitology and serology tests in the suspected animal reservoirs

Type of animal	No. of animals	Parasitology results (no. of positive cases)	Serology results (no. of positive cases)		
			DAT	ELISA	IFA
Leashed dogs	97	3	3	2	2
Stray dogs	8	0	1	0	0
Foxes	4	0	2	0	0
Jackals	10	1	1	1	1
Rodents	193	0	0	0	1

DAT = direct agglutination test. IFA = immunofluorescence assay.  
ELISA = enzyme-linked immunosorbent assay.

er, the GMRT in females (15848.9) was very much higher than in males (5214.8).

Out of 18 diagnosed cases of kala azar found here, 89% were children less than 4 years old and all of them were under 9 years. All of them were seropositive ( $\geq 1:3200$ ) by DAT. Fever and splenomegaly were the predominant clinical features. These signs and symptoms are the same as those found in other clinical studies [2,8]. Examination of the history of kala azar patients showed that kala azar cases are much more frequent in nomads, and most seropositive cases were found among nomadic children.

The animal reservoir hosts were found in this investigation to be the *Canidae* family (dog, jackals). On the basis of our results, this endemic focus of VL in Bushehr province is similar to the endemic focus of Jahrum, Ghir and Firooz Abad in Fars province and other endemic areas of the Islamic Republic of Iran where the kala azar is of the Mediterranean type [10].

To control VL in this area, we suggest the following measures: eliminating stray dogs; identifying suspect leashed dogs by

periodic DAT serological tests and exterminating those found infected; and identifying human cases using practical serological tests such as DAT and treating infected individuals in order to decrease the mortality rate. Nevertheless, further investigations are needed to discover the main vector of the disease in this area.

## Acknowledgements

This study was supported by the Institute of Public Health Research, Teheran University of Medical Sciences. We wish to thank Dr A. Nadim for advice and critical comments, Dr Y. Rassi and his colleagues in training at the Health Research Centre of Kazerun, Dr M.H. Motazedian for performing RAPD-PCR in the Shiraz University of Medical Sciences, and Mr S.H. Hajjaran for providing the DA antigen. Also we are grateful for the sincere cooperation of the directors and staff of the health and medical centres as well as the environmental protection agencies in Dashti and Dash-testan.

## References

1. Edrissian GH et al. An endemic focus of visceral leishmaniasis in Meshkin Shahr, east Azerbaijan province, north-west part of Iran and IFA serological survey of the disease in this area. *Bulletin de la société de pathologie exotique et de ses filiales*, 1988, 81(2):238-48.
2. Edrissian GH et al. Serological studies of visceral leishmaniasis and search for animal reservoirs in Fars province, southern Iran. *Iranian journal of medical sciences*, 1993, 18(3):99-105.
3. Edrissian GH et al. Visceral leishmaniasis: the Iranian experience. *Archives of Iranian medicine*, 1999, 1(1):22-6.
4. El-Harith A et al. Improvement of direct agglutination test for field studies of visceral leishmaniasis. *Journal of clinical microbiology*, 1988, 26(7):1321-5.
5. El-Harith A et al. Application of direct agglutination test for detection of specific anti-*Leishmania* antibodies in the canine reservoir. *Journal of clinical microbiology*, 1989, 27(10):2252-6.
6. Noyes HA et al. Appraisal of various random amplified polymorphic DNA-polymerase chain reaction primers for *Leishmania* identification. *American journal of tropical medicine and hygiene*, 1996, 55(1):98-104.

7. Motazedian H et al. Leishmania and sauroleishmania: the use of random amplified polymorphic DNA for the identification of parasites from vertebrates and invertebrates. *Experimental parasitology*. 1996. 83(1):150-4.
8. Soleimanzadeh G et al. Epidemiological aspects of kala azar in Meshkin-Shahr, Iran: human infection. *Bulletin of the World Health Organization*, 1993, 71(6):759-62.
9. Boelaert M et al. Cost-effectiveness of competing diagnostic-therapeutic strategies for visceral leishmaniasis. *Bulletin of the World Health Organization*, 1999, 77(8):667-74.
10. *Control of the leishmaniases: report of a WHO expert committee*. Geneva, World Health Organization, 1990:10-2 (WHO Technical Report series, No. 793).

### **WHO report on global surveillance of epidemic-prone infectious diseases**

This report focuses on the analysis and interpretation of data collected by WHO on the surveillance of infectious epidemic diseases, the strengths and weaknesses of the data, and how the data can be used and interpreted. This volume uses a multiple disease approach, and examines not only the surveillance of (nine) different diseases, but also contrasts and compares their global surveillance systems. The nine infectious epidemic diseases covered are either new or volatile or pose an important public health threat. All have high epidemic potential and most are increasing in incidence. They include: yellow fever, plague, cholera, meningococcal disease, dengue fever and dengue haemorrhagic fever, influenza African trypanosomiasis, HIV/AIDS, leishmaniasis and leishmania/HIV co-infection. These diseases are difficult to track because of their complicated epidemic patterns, their ability to develop new strains, and their tendency to spread quickly to new locations. Most of these diseases have high case fatality rates and severe symptoms increasing the urgency of fast identification of new occurrences to prevent further transmission. This report can be obtained from: Department of Communicable Disease Surveillance and Response, World Health Organization, Avenue Appia 20, CH-1211 Geneva 27, Switzerland. It is also available free on the Internet at: <http://www.who.int/emc-documents/surveillance/docs/whocdscsrir2001.pdf/index.html>