A new epidemic focus of zoonotic cutaneous leishmaniasis in central Iran

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Background: Reports from the health center of Yazd province of increasing cutaneous leishmaniasis (CL) cases led us to carry out an epidemiological study using standard techniques in Ardakan County, central Iran, during 2001.

Patients and Methods: Data was collected on the prevalence of scars and ulcers over a period of 14 months among 621 households in three villages around Ardakan County. Smears were prepared by scraping the edges of the ulcers. We collected the same data on all school children aged 7 to 11 years old in the area. To determine the reservoir host of the disease, rodents and dogs were caught and examined. Sandflies were collected biweekly from indoor and outdoor locations in the study area, and then identified. Parasites isolated from human and rodents were characterized by RAPD-PCR technique.

Results: The prevalence of scars and ulcers were 30.4% and 24.6%, respectively, in 3024 individuals in the three villages. Individuals 10 to 14 years of age were the most highly infected age group, with a rate of 28.4%. Males and females were equally infected. Examination of 892 students in primary schools showed a rate of 22.9% for scars and 23.7% for ulcers. *Meriones libycus* (42.2%) and *Rhombomys opimus* (57.8%) were present around the villages. Both were infected with *Leishmania*. Three of 19 *M. libycus* (15.7%) and 3 out of 26 *R. opimus* (11.5%) had positive results. The active season of sandflies was late April to late November. *Phlebotomus papatasi* and *Sergentomyia sintoni* were the dominant species indoors and outdoors. Natural leishmaniasis infection was found in *P. caucasicus* and *S. sintoni* from gerbil and jird burrows.

Conclusion: Based on this survey, there is an epidemic of zoonotic CL in the area, with *Leishmania major* as the agent, *M. libycus* and *R. opimus* as the reservoir hosts, and most probably *Phlebotomus papatasi* as the vector because about 77% of indoor sandflies were of this species.

Key words: Cutaneous leishmaniasis, *Leishmania major*, epidemic, zoonosis, epidemiology, Iran

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Accepted for publication: January 2004

Ann Saudi Med 24(2):98-101

Zoonotic cutaneous leishmaniasis is common in many rural areas of Iran, having been reported in 13 of the 28 provinces.¹ In some parts of Yazd province in central Iran, the number of reported cases of cutaneous leishmaniasis has increased since 1981. From 1993 to 1997, a total of 2393 cases were officially reported in Yazd (mean annual population of Yazd, 736 576), but this was probably a large underestimate. The disease is widespread in seven counties of the province, but Ardakan is the most important focus in the area. During 1996 to 1997, a total of 372 cases were officially reported, all by passive case detection (health center of Yazd province, unpublished data).

Cutaneous leishmaniasis (CL) is a serious and increasing public health problem in the area. Cases from Ardakan County are diagnosed more and more frequently in health centers and local clinics in the cities of Ardakan and Yazd.

However the epidemiological aspects of CL in the area have not yet been examined. Therefore, we carried out an epidemiological investigation in this new focus for the purpose of implementing control measures in the future.

Materials and Methods

The investigation was conducted over a period of 14 months, from 4 April 2001 to 4 July 2002, in three villages (Chahafzal, Torkabad and Ahmadabad, population 7058), located 5 to 30 km west of Ardakan city, central Iran. The basis for choosing the villages was a preliminary report of increased cases by the health center of Yazd province. The soil of the area is soft clay and generally salty. However, pistachio, pomegranate, wheat, barley, beetroot, turnip, alfalfa, alizarin and cotton are cultivated. The underground water table is about 20 meters deep in this area (data from General Office of Agriculture, Yazd province), which has a

desert climate, and is very hot in summer and cold in winter. In 2001 the maximum and minimum mean monthly temperature was 44.6 °C and -3.3 °C in July and January, respectively. The total annual rainfall was 108.9 mm, ranging from 0.5 mm in May and September to a maximum of 38.3 mm in December. The minimum mean monthly relative humidity was 23% (July) and the maximum was 62% (January).

Population studies. All the inhabitants of Chahafzal and Torkabad and 150 households of Ahmadabad whose buildings were located near each other (the village had 923 households) were examined in late November 2001, and forms were completed for each household during a house-to-house visit. The presence or absence of scars or ulcers of CL was indicated on the form. We also examined and questioned all the students of elementary schools in the surveyed area. Each individual was examined for scar(s) or ulcer(s), and the date and place of acquiring the disease, age, sex, number of ulcers or scars, site of ulcer(s) or scar(s) was recorded. Study personnel differentiated CL from other scars by taking into consideration that the CL ulcer heals gradually, taking weeks to years, and leaves a depressed scar with altered pigment. It has a distinct limit with an irregular margin and a rough surface with small hollows and swellings. Smears were prepared from scrapings of the edge of the ulcer, then fixed in methanol, stained with Giemsa, and examined under a light microscope for the presence of amastigotes. The χ^2 -test was used to determine statistically significant differences in disease prevalence between females and males and among different age groups in the community and school children.

Collection and examination of rodents and dogs and parasite isolation from rodents. Rodents were caught in 20 live traps, which were baited with cucumbers and dates once a month. In the laboratory, six impression smears were prepared from the ears of each rodent,² then fixed in methanol, stained by the standard Giemsa method, and examined carefully under the microscope. Stray dogs were shot and physically examined for the presence of any ulcer or scar.

Samples from infected rodents were injected subcutaneously in the base of the tail of inbred (BALB/c) mice to identify the parasite. Parasites were reisolated from infected mice and cultured in NNN media plus LIT medium containing 200 IU penicillin per mL, incubated at 20 to 21 °C and monitored every 4 days, from day 4 for growth. Cultures were examined for 6 weeks before being considered as having no growth. All positive cultures were subcultured every 15 days. Cultured promastigotes isolated from rodents were sent to the Faculty of Medicine, Shiraz University of Medical Sciences (Prof. S. Ardehali and Dr. M.H. Motazedian) for further identification using the Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR).^{3,4}

Isolation of parasites from patients. Samples from six patients (who were surely infected in the study villages) were taken and inoculated subcutaneously at the base of the tail of 18 BALB/c mice. Parasites were reisolated from infected mice, cultured and examined as previously described for rodents. RAPD-PCR technique is used for identification of parasites at the Protozoology Unit, Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences (Dr. M. Mohebbi and Dr. M.H. Motazedian).

Collection of sandflies. Sandflies were collected biweekly from indoor (bedrooms, warehouses, privacies, etc) and outdoor (rodent burrows) fixed locations in the study area using 30 sticky traps (caster oil coated white papers 20x32 cm) from the beginning (April) to the end (October) of the active season. For species determination, sandflies were mounted in Puri's medium⁵ and identified after 24 hours using the keys of Theodor and Mesghali,⁶ and then counted and segregated by sex.

Results

In the population of 3024 persons from 621 households in the three villages, the prevalence of scars was 30.4%. The scar rate was 22.8% for individuals under 10 years of age and 31.9% for those over 10 years of age. There was a significant difference between males and females ($\chi^2=22.52$, $df=1$, $P<0.01$). The proportion of males and females were 51.2% and 48.8% respectively. Significant differences in number of individuals with scars were also observed among different age groups ($\chi^2 = 25.785$, $df = 5$, $P<0.01$). The infection rate increased up to the age of 14 years and from 14 years upwards remained more or less the same (Table 1).

The prevalence of ulcers (active lesions) among the inhabitants was 24.6%. The most highly infected age group was 10 to 14 years, which had an active lesion rate of 28.4%. Non-significant differences were observed between infected males and females among local residents ($\chi^2 = 1.25$, $df = 1$, $P>0.05$). Children under 10 years of age had a rate of 20.9% for ulcers. The rate was 25.3% for those more than 10 years old. The hands, legs and face were the most affected parts of the body, with 49.3%, 35.8% and 8.1% of the active lesions, respectively; 46.8% of the patients had one, 22.7% had two and 30.5% had ≥ 3 ulcers. The youngest case was a 3-month old boy and the oldest, a 95-year old woman. Parasites from six patients with ulcers were injected subcutaneously at the base of the tail of 18 BALB/c mice. In four (22.2%) mice, nodules and ulcers containing numerous amastigotes appeared at the site of inoculation 65 to 72 days after injection.

Altogether, 7 primary schools with 892 students (447 boys and 445 girls) from 7 to 11 years of age were visited in November 2001. The overall scar rate was 22.9% and the

Table 1. The prevalence of cutaneous leishmaniasis by age among 621 families (both sexes) in the study area, November 2001.

Age group (y)	No. observed	No. with scars	%	No. (%) with active lesions	%
0-4	190	29	15.3	42	22.1
5-9	311	85	27.3	63	20.3
10-14	524	158	30.2	149	28.4
15-19	530	170	32.1	131	24.7
20-24	301	98	32.6	65	21.6
25+	1168	380	32.5	293	25.1
All groups	3024	920	30.4	743	24.6

Table 2. The prevalence of active lesions (ulcers) and scars by age among students of primary schools (both sexes) in the study area, November 2001.

Age (y)	No. observed	No. with scars	%	No. with active lesions	%
7	146	33	22.6	22	15.1
8	169	32	18.9	45	26.6
9	164	39	23.8	31	18.9
10	173	42	24.3	44	25.4
11	240	65	27.1	69	28.8
Total	892	205	22.9	211	23.7

prevalence of ulcers was 23.7% (Table 2). In children with active lesions, 46.9% had only one ulcer, 21.3% had two and 31.8% had three or more than three ulcers. Non-significant differences were observed in the prevalence of active lesions by sex between individuals younger and older than 10 years of age ($\chi^2 = 0.87$, $df = 1$, $P > 0.05$). All patients had contracted the disease in 2001 in their villages. The hands, legs and face were the most affected parts of the body, with 45.3%, 33.3% and 12.7% of the active lesions, respectively.

All slides prepared by scraping the edges of ulcers of all patients (inhabitants of households and primary schools) contained parasites presumed to be *L. major* based on the existence of a large vacuole in the cytoplasm. Treatment was provided for 954 subjects with a parasitological diagnosis of leishmaniasis.

A few colonies of rodents were found around Chahafzal and Ahmadabad, but there were many active colonies of *Rhombomys opimus* around the village of Torkabad. Nineteen *Meriones libycus* and twenty-six *R. opimus* were collected and examined carefully around the study villages. Both *M. libycus* and *R. opimus* were found to be infected with *Leishmania*. Three of 19 *M. libycus* (15.7%) and three of 26 *R. opimus* (11.5%) were positive. Parasites from one infected *M. libycus* were injected subcutaneously into 5 BALB/c mice. Nodules and ulcers containing numerous amastigotes appeared within 4 months in 2 mice, and within 5 days cultures from the lesions contained profuse promastigotes. None of six stray

dogs examined around the villages appeared to be infected.

During April to November 2001, 4191 adult sandflies (3901 from outdoors and 290 from indoor resting places) were collected and identified. The following four species were found in bedrooms, warehouses and privacies: *P. papatasi* (76.9%), *P. mongolensis* (1.4%), *P. causicus* (0.7%) and *Sergentomyia sintoni* (21%). In rodent burrows, cracks in walls and bird holes, *P. papatasi* (42.5%), *P. mongolensis* (1.3%), *P. causicus* (0.7%), *S. sintoni* (53.6%), *S. dentata* (1.5%) and *S. antennata* (0.4%) were collected. Common sandflies in indoor and outdoor resting places were *P. papatasi* and *S. sintoni*. The sandflies started to appear in April and disappeared in November. There were two peaks in the density curve of these two species in the area, one in late May and the second in the middle of August.

The sex ratio, i.e., number of males per 100 females of *P. papatasi*, was found to be 147.8 and 112.3 in outdoors and indoors, respectively. In September 2001, *P. papatasi* (41), *P. causicus* (1) and *S. sintoni* (25) were collected in the vicinity of rodent burrows and were dissected. The results of these dissections showed that *P. causicus* and

S. sintoni (4%) were infected with leptomonads. We dissected 76 *P. papatasi* collected from indoors at the same time. All were negative.

Discussion

This is the first time that an epidemic focus of cutaneous leishmaniasis due to *Leishmania major* has been identified in Ardakan County, central Iran. *L. major* has a wide distribution from the sub-Saharan Sahel to the near and Middle East.⁷ We have isolated this parasite from *P. papatasi*, *P. salehi*, *P. caucasicus*, *M. libycus*, *R. opimus*, *Tatera indica* and humans in other parts of Iran.^{8,9,10,11}

The prevalence of CL among the households (30.4 % for scars and 24.6% for ulcers) was higher than that seen for school students. A prevalence of 32.5% of *Leishmania* scars among people older than 25 years was due to the lack of awareness of the local people about early symptoms of the disease and recognition of the need to seek medical assistance. This situation has been continuing for years during which time there has been no health education program in the area.

Our findings suggest that *M. libycus* and *R. opimus* are the main reservoir hosts of zoonotic CL and the principal source of human infection in the area west of Ardakan city. *M. libycus* has been reported as the major reservoir host of zoonotic CL in other endemic areas near Ardakan County, such as Ardestan and northwest of Natanz, and *R. opimus* appears to be the main reservoir host in Isfahan, Zavvareh and east of Natanz (the approximate distance of these foci from Ardakan are about 200 km). Six species of sandflies (three species of genus *Phlebotomus* and three species of *Sergentomyia*) were identified. *S. antennata* is new in the area and was not reported in a previous study conducted in 1969 (Institute of Public Health, unpublished data).

Based on this survey, there is an epidemic of zoonotic CL around Ardakan, with *L. major* as the agent, *M. libycus* and *R. opimus* as the reservoir hosts, *P. caucasicus* and *P.*

papatasi as the vectors among rodents, and probably *P. papatasi* as the vector to man because about 77% of indoor sandflies were of this species. This new focus should be added to the list of zoonotic CL foci in Iran. Over the last decade, cases of zoonotic CL have been reported mostly from the west and southwest of the near central desert (Badrood, Ardestan, Zavvareh, Abouzaidabad, Abarkooh) and even from the south of the country (Neiriz, Estahban, Lar, Darab, Jahrom, Kharameh, Sarvestan and Arsanjan). Ardakan is also an extension of these foci. The occurrence of this outbreak of zoonotic CL in the study villages seems to be the result of 1) the construction of buildings in farms near the colonies of rodents, 2) the habit of sleeping on flat roofs and in yards during the summer, and 3) agricultural development taking place in the area.

It is essential for the health authorities to take strong steps to control the epidemic and prevent its spread to neighboring villages by destroying gerbils with zinc phosphide mixed with wheat grains and vegetable oil (2.5%) within a radius of 500 meters once a month during May, June, July, and September. It is also essential to provide rapid treatment of patients in the coming years. The control of zoonotic CL ultimately rests on close cooperation between the universities of medical sciences, health centers and the government.

Acknowledgements

We are grateful to the leishmaniasis staff in the Yazd and Isfahan Training and Public Health Research Centers for assistance in carrying out this program. Thanks are also due to Dr. M. Mohebbali and Dr. M.H. Motazedian for their assistance in the confirmation of the identity of *L. major* and Prof. E. Javadian and Dr. M. Karimi for their help and support with this study. This work has received financial support from Institute of Public Health, Tehran University of Medical Sciences (Project No. 241.77.6).

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