

# A new focus of cutaneous leishmaniasis caused by *Leishmania tropica*

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## ABSTRACT

**Objective:** To determine the epidemiological status of the cutaneous leishmaniasis outbreak, isolation and identification of the parasite and preparation of a control program in a recently identified focus located in the Province of Yazd, Central Iran.

**Methods:** This study was conducted in 124 classrooms of primary school children and among 139 households of an infected quarter in the city of Yazd during 1999-2000. All the students and members of the households were questioned and examined for the presence of ulcer(s) or scar(s). For each case a form was completed and necessary information were recorded. Smears were prepared from the edge of the ulcer and examined for the presence of amastigotes. *Leishmania* stocks from human lesions were isolated. Cultured promastigotes were identified using Random Amplified Polymorphic DNA-Polymerase Chain Reaction. Stray dogs were shot in the city and examined for the presence of any ulcer(s) or scar(s).

**Results:** An endemic focus of cutaneous leishmaniasis caused by *Leishmania tropica* was detected in Yazd City, Central Iran. Examination of 3176 students aged 6-16 years old in 124 primary schools showed a rate of 0.7% for

scars and 0.2% for active lesions. There was no significant differences between the sexes in the prevalence of active lesions or scars. A study of prevalence among 139 households with a total population of 572 persons in Darvazeh-Ghassabha quarter showed a prevalence of 13.5% for scars and 0.5% for ulcers. The most highly infected age group was 5-9 with a rate of 1.8%. Males and females were equally infected. Forty-six stray dogs were examined and none of them appeared to be infected.

**Conclusion:** According to this study, cutaneous leishmaniasis due to *Leishmania tropica* is endemic in the city of Yazd, Central Iran. Humans are considered to be the main reservoir and the transmission is believed to be from human to human by *Phlebotomus sergenti*. This focus now can be added to the list of anthroponotic cutaneous leishmaniasis foci in the country. Any residual insecticide spraying is not recommended during the next year due to the low prevalence of the disease.

**Keywords:** Epidemiology, cutaneous leishmaniasis, *Leishmania tropica*.

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Leishmaniasis is a zoonoses with a variety of mammalian reservoir hosts, including rodents. After malaria, leishmaniasis is probably the 2nd most important protozoan disease. It is transmitted naturally by the bite of an infected sandfly. The distribution of Old world cutaneous leishmaniasis (CL) includes the Middle East. Clinical

manifestations include a primary lesion which evolves into a shallow ulcer with raised borders. Lesions are commonly located on exposed areas of the face and extremities and may be associated with satellite lesions and regional adenopathy. Resolution of lesions may take from weeks to years and results in a flat atrophic scar. Anthroponotic cutaneous

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leishmaniasis (ACL) has been a very old endemic disease in many parts of our country. It was greatly reduced in many foci by beginning antimalarial measures in Iran but its foci remained active in some large and medium sized cities such as Tehran, Mashhad, Neishabur and Sabzevar in the northeast, Shiraz in the south, Kerman and Bam in the southeast and parts of the city of Isfahan in the central region.<sup>1-6</sup> Since 1981, the reported cases of CL have increased in some parts of Yazd Province. From 1993 to 1997 a total of 2393 cases were officially reported but this is probably a large underestimate. One of these foci is the city of Yazd. In recent years, ACL has become the most important endemic disease in this city and a matter of concern for health authorities. From 1995 to 1997 a total of 543 cases were officially recorded, all by passive case detection (Health Center of Yazd Province). There was no accurate data on the prevalence of the disease in this focus, therefore, a cross-sectional descriptive study of the disease was carried out during 1999-2000 for the preparation of the control program.

**Methods. Study area.** The city of Yazd (31° 52'N, 54° 22'E), the center of Yazd Province is located in the central part of the country. Its altitude is 1212 meters above the sea level. The city had a population of 352,800 in 1999 while this was only 135,925 in 1976 with an increase of more than 2.5 folds in the last 2 decades. The city has a desert climate, the summers are hot and arid and the winters are cold and arid. In 1999 the maximum and minimum temperature were 45.8°C in August and 1.6°C in February. The total annual rainfall was 114.4mm and the relative humidity varied between 17-42% in the same year.

**Population studies.** The study has been carried out in 2 population groups during 1999-2000. 1. Primary school children 2. The population in an infected quarter of the city (for obtaining data on human infection rate in all age groups). For the first group a list of all primary schools in Yazd was obtained from the Department of Education. Then, 124 classrooms were selected by cluster method. Each class was visited and a list was prepared from all the students and they were questioned and examined for the presence of ulcer(s) or scar(s). For each case having ulcers or scars, a form was completed to record the necessary information such as name, address, age, sex, number of ulcer(s) or scar(s), site of ulcers or scars, date and place of acquiring the disease, and so forth. Smears were prepared from the edge of the ulcer fixed in methanol, stained with Giemsa and examined under a light microscope for the presence of amastigotes. All the students were visited in November 1999. The  $\chi^2$  and Fisher tests were used to determine any statistically significant differences in disease

prevalence between female and male school children between, under and above 10-years old (males and females).

For the 2nd group, one part of the city was selected, Darvazeh-Ghassabha, approximately near the center of the city from which most active cases of CL were recorded by the health centers. Buildings are made of brick and mud in this quarter. One hundred and thirty-nine households whose buildings were located near each other were visited and all members of the households examined and special forms were filled out, the same as described for the students.

**Isolation of parasites from patients.** A sample from 7 patients (who were infected in the city) were inoculated into Novy-Nicolle-MacNeal (NNN) medium plus liver infusion tryptose (LIT) medium containing 200 iu penicillin per ml, incubated at 20-21°C and monitored every 4 days, from day 4 for growth. Cultures were examined for 6 weeks before being considered no growth. All positive cultures were subcultured every 15 days. Cultured promastigotes isolated from 5 patients (7-62 years of age) were sent to the Faculty of Medicine, Shiraz University of Medical Sciences for further identification using Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR).

**Collection and examination of dogs.** Stray dogs were shot in autumn 2000 in different parts of the infected quarters in the city and examined carefully for the presence of any ulcers or scars.

**Results.** A total of 3176 children aged 6-16 years in 124 primary schools, comprising 1591 girls (50.1%) and 1585 boys (49.9%) were examined physically for the presence of active lesions or scars of CL. The overall scar rate was 0.7% and the prevalence of ulcers was 0.2% (**Table 1**). Non-significant differences were observed in the prevalence of CL by sex ( $P < 0.05$ ). The higher and lower prevalence were calculated 1.3% and 0.13% in  $\geq 11$  and 9 age groups. The Fisher test showed non-significant differences in the prevalence of CL by sex among those aged under and above 10 years ( $P > 0.05$ ). In children with active lesion, 71.4% had one lesion, 14.3% had 2 lesions and 14.3% had 3 lesions. The average number of lesions was 1.28. Face and hands were the affected parts of the body with 44% and 56% of the active lesions. All slides which have been prepared by scraping the edges of ulcers of all patients contained parasites morphologically resembling *Leishmania tropica* (numerous parasites, small in size, oval shape, without vacuole). Treatment was provided for the 7 subjects with parasitologically diagnosis of leishmaniasis. A study of prevalence among 139 households with a total population of 572 persons in Darvazeh-Ghassabha quarter showed 13.5% for scars

**Table 1** - Prevalence of cutaneous leishmaniasis by age and sex in primary school children in the central Iranian city of Yazd, November 1999.

Age (year)	N observed	N with scars (%)	N with active lesions (%)
6	150	0 (0)	0 (0)
7	515	2 (0.38)	0 (0)
8	668	3 (0.45)	2 (0.29)
9	789	4 (0.5)	1 (0.13)
10	756	4 (0.53)	0 (0)
>11	298	8 (2.68)	4 (1.34)
<b>Total</b>	<b>3176</b>	<b>21 (0.66)</b>	<b>7 (0.22)</b>
N - number			

and 0.5% for ulcers, both are higher than what was seen in school students. The highest rate for those having scars was 27.8% in the age group 15-19 years and the lowest rate was 9.3% in the age group > 25 years. The scar rate was 10.2% for individuals under 10 years of age and 14.04% above 10 year old. Males and females were equally infected. Prevalence of ulcers among the inhabitants was 0.5%. The most highly infected age group was 5-9 with a rate of 1.8%. Non-significant differences were observed between infected males and females ( $P < 0.05$ ). No active cases were seen under 4 years of age. The ulcer rate was 1.2% for individuals under 10 years of age and 0.4% above 10 year old (**Table 2**). Fifty percent of the patients had one and the rest had 2

ulcers. Face and hands were equally infected. The average number of lesions was 1.5. The youngest case was 10-year- old and the oldest was a 50-year-old woman. Examination of isolates from 5 patients showed that the isolates from human indigenous cases in Yazd were *Leishmania tropica*. Forty-six stray dogs were examined and none of them appeared to be infected.

**Discussion.** This is the first time that an endemic focus of CL due to *Leishmania tropica* was identified in the city of Yazd, central Iran, where ACL has been present in recent years. *Leishmania tropica* has a wide distribution from the Mediterranean, through the Middle East and central Asia, to the west of India and is probably an anthroponotic infection in most areas.<sup>7</sup> Examination of several isolates from human has revealed that the causal organism was also *Leishmania tropica* in the cities of Shiraz, Tehran, Bam and Isfahan in our country.<sup>8-11</sup> The distribution map of active cases in the city suggests that most of the cases belong to the central part where there are lots of old mud houses which are made of sun-dried bricks and they are located along 2 old ruined subterranean canals. The land is suitable for breeding of sandflies capable of being vectors of CL in this part of the city.

The present investigation revealed the disease is in endemic form with low endemicity, otherwise we would have seen higher scar rates. Most probably, *Phlebotomus sergenti* is the vector as 75% of indoor sandflies were of this species. Humans are considered to be the main reservoir and transmission is believed to be from human to human.

The occurrence of this outbreak of ACL in the city of Yazd seems to be the result of movement of infected people and an increase in the human and the

**Table 2** - Prevalence of cutaneous leishmaniasis, in the quarter of Darvazeh – Ghassabha, Yazd city, by sex and age, October - November 1999.

Age group (years)	N observed			N with scar			Prevalence of scars (%)			N with AL			Prevalence of AL (%)		
	M	F	Total	M	F	Total	M	F	Total	M	F	Total	M	F	Total
0-4	18	15	33	0	0	0	0	0	0	0	0	0	0	0	0
5-9	28	27	55	4	5	9	14.3	18.5	16.4	0	1	1	0	3.7	1.8
10-14	34	48	82	7	9	16	20.6	18.8	19.5	1	0	1	2.9	0	1.2
15-19	39	33	72	9	11	20	23.1	33.3	27.8	0	0	0	0	0	0
20-24	37	25	62	5	2	7	13.5	8.0	11.3	0	0	0	0	0	0
≥ 25	138	130	268	11	14	25	7.9	10.8	9.3	0	1	1	0	0.8	0.4
All ages	294	278	572	36	41	77	12.2	14.8	13.5	1	2	3	0.3	0.7	0.5
N - number, M - male, F - Female, AL - active lesions															

vector population (*Phlebotomus sergenti*), a phenomenon noticed in many places in Iran as well as other countries of the region. Any residual insecticide spraying is not recommended during the next year due to the low prevalence of the disease. The impossibility of complete spraying coverage is due to the existence of a great number of houses in the city and resistance shown by the majority of the target population.

If the present epidemiological situation for breeding of the vector are changed (example setting up big gardens in different parts of the city, and so forth) and the disease becomes epidemic in the future, chlorophenothane (DDT) spraying in the houses with active cases and also all their neighboring houses and in all places with high density of *Phlebotomus sergenti* should be applied only once during the active season of sandflies. Infected stray dogs should be found and eliminated, if any. Active and passive case detection and rapid treatment are also recommended. Intensive health education programs have to be strongly supported in order to promote awareness among the exposed population.

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