

# **Epidemic Outbreak of Cutaneous Leishmaniasis due to *Leishmania major* in Ghanavat Rural District, Qom Province, Central Iran**

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## **Abstrat**

An outbreak of zoonotic cutaneous leishmaniasis caused by *Leishmania major* was identified in Ghanavat rural district, Qom province, central Iran, during 1999-2001. Among 1069 inhabitants examined in Dec. 1999, 5.14% showed evidence of active lesions and 12.44% had scar indicative of past infection. The most highly infected age group was 5-9 years old for ulcers with a rate of 6.56%. The incidence of the disease was calculated as 2.7% and 1.4% in 2000 and 2001 respectively. *Meriones libycus* (66.7%), *Nesokia indica* (27.3%) and *Hemechinus aurithis* (6%) were present around the district. No leishmanial infection was seen in the slides. *Meriones libycus* is the probable reservoir host of the disease in the area. Sixteen dogs appeared to be uninfected because examination showed no active lesion or scar. Most probably *Phlebotomus papatasi* is the vector because 81.53% of indoor sand flies were of this species. The active season of *P.papatasi* was from late April to early October in indoors. The occurrence of this outbreak of ZCL in the district seems to be the result of construction of buildings near colonies of rodents and also traveling to other infected foci of Iran.

**Keywords:** *Leishmania major, Leishmaniasis, outbreak, Sand flies, Reservoirs, Host, Iran*

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## **Introduction**

Zoonotic cutaneous leishmaniasis (ZCL) is a major and increasing public health problem in 11 of the 28 provinces of Iran (1). Recently a new focus of cutaneous leishmaniasis (CL) has been found in some villages of Ghanavat rural district, 30 km from the city of Qom, one of the most important religious cities in Iran. In 1998, a total of 249 cases (124 from the city and 125 from rural districts) were officially reported by passive case detection (Qom Health Center, Unpublished data). The infected area is located

close to the Jamkaran holy mosque and lots of people from all provinces of the country visit it during the active season of sand flies. However the epidemiological aspects of cutaneous leishmaniasis in this district have not been examined yet. Therefore in depth epidemiological survey was carried out for the first time in this focus for implementation of future control measures.

## Materials and Methods

The investigation was conducted over a period of 27 months from November 1999 to March 2002 in Ghanavat rural district of Qom province (50-52 ° E, 34-35° N), 30 km southeast of Qom city, the center of the province, central Iran. The area has a desert climate, hot in the summer and quite cold in the winter. In 2000, the maximum and minimum mean monthly temperatures were 32.4°C and 4.1°C in July and February respectively.

The total annual rainfall was 193.8 mm with a minimum of 2.6 mm in April and maximum of 80.6 mm in December. The minimum mean monthly relative humidity was 24% (June) and the maximum was 85.3% (November). Studies on human infection have been carried out in two population groups: 1- All students of the School of Hossein-abad Mishmast village where most active cases of CL were recorded by Qom Health Center. 2- The population of the infected villages (to obtain data on human infection rate in all age groups). For the first group, a list of all of the schools in the village of Hossein-abad Mishmast was obtained. Each school was visited and in each class, a list was prepared from all of the students, then they were questioned and examined for presence of ulcer(s) or scar(s).

For each case having these signs, a form was completed to record the necessary information such as names, address, age, sex, number and sites of ulcer(s) and scar(s), date and place of acquiring the disease, etc. All of the students were visited once a season except summer when the schools were closed. For the second group, three villages called Hossein-abad Mishmast, Djannat- abad and Dowlat-abad were selected to determine the prevalence rate of the disease in November 1999.

To determine the incidence of the disease in 2000 and 2001, the village of Hossein-abad Mishmast where the most active cases of CL were recorded by Qom Health Center, was selected. Samples from 3 patients were taken

from the sores and were inoculated subcutaneously at the base of the tail of BALB/c mice (each for 2 mice). Parasites were reisolated from infected mice and cultured in NNN plus LIT medium containing 200 IU penicillin per ml, incubated at 20-21 °C and monitored every 4 days, from day 4 for growth. All positive cultures were subcultured every 15 days in RPMI medium containing 10-20% FCS. After growing the parasites, leptomonads in logarithmic phase were cryopreserved in -196 °C and then in proper time RAPD-PCR technique with the appropriate primers was used for identification of parasites in Protozoology unit, Department of Medical Parasitology, School of Public Health, Tehran University of Medical Sciences, with the cooperation of School of Medicine, Shiraz University of Medical Sciences (2, 3).

Small mammals caught by 56 live traps baited with cucumber and tomato, once a month from March to February, 2000 and by 32-40 live traps from June to August 2001.

In the laboratory, 4 impression smears were prepared from the ears of each mammal (4) fixed in methanol and stained by the Geimsa method and examined carefully under the light microscope. Furthermore 16 household dogs were physically examined for the presence of any ulcer or scar in the village of Hossein-abad Mishmast. Sand flies were collected biweekly from indoor (bed rooms, warehouse, etc.) and outdoor (rodent burrows) fixed places in Hossein-abad, using 30 sticky traps (castor oil coated white paper 20 x 32 cm) from the beginning (May) to the end (November) of the active season. For species identification, sand flies were mounted in Puri's medium (5) and identified after 24 h using the keys of Theodor and Mesghali (1964).

Then they were counted and segregated by sex. In order to determine natural leptomonad infections of sand flies, some unfed, blood fed, semigravid and gravid female sand flies of rodent burrows were collected by sticky traps and examined in a fresh drop of sterile saline

(9/1000) for the presence of promastigotes in alimentary canal. The data were analyzed using EPI-6 package ( $\chi^2$  test) and the graphs were prepared by Excel<sup>®</sup>.

## Results

A study of prevalence among the inhabitants of Hossein-abad Mishmast village (1069 persons), Djannat-abad (1367 persons) and Dowlat-abad (203 persons) showed 12.44%, 1.1% and 0.99% rate for scars and 5.14%, 0.8% and 1.97% for ulcers respectively. In Hossein-abad Mishmast, the most highly infected age group for ulcer was 5-9 years old with a rate of 6.56% but the least infected age group was 15-19 years with a rate of 4.52 (Table 1).

Sixty percents of the patients had one, 16.4% two and 23.6% three or more ulcers. Out of 62 ulcers, 51.6% were located on hands, 29% on feet, 12.9% on face, 1.7% on neck and 4.8% on the other parts of the body. In order to determine the incidence of the disease in 2000 and 2001, all of the inhabitants were visited once each season and only new cases were recorded.

The incidence of the disease was calculated as 2.7% and 1.4% in 2000 and 2001 respectively. The *Leishmania* parasites were isolated from ulcers of inhabitants with no traveling history to other foci of the disease and identified as *Leishmania major* by RAPD-PCR method. In the school survey, 179 students (60 girls and 119 boys) in 2000 and 173 students (57 girls and 116 boys) in 2001 were visited and examined. The incidence rate of ulcers was calculated 2.79% and 2.26% respectively. Sixteen domestic dogs were examined and none

of them appeared to be infected. Some colonies of rodents were found around the villages. A total of 33 small mammals including *Meriones libycus* (66.7%), *Nesokia indica* (27.3%) and *Hemiechinus aurithis* (6%) were collected and examined carefully. No leishmanial infection were seen in the slides.

A total of 7425 adult sand flies (222 from indoors and 7203 from outdoors) were collected and identified during April-October 2000.

The following two species were found in indoors: *Phlebotomus papatasi* (81.53%) and *Sergentomyia sintoni* (18.47%). *Phlebotomus papatasi* (2.33%), *S. sintoni* (95.22%), *S. dentata* (2.42%) and *S. clydei* (0.03%) were also collected from outdoors. The most common sand flies in indoors and outdoors resting places were *P. papatasi* and *S. sintoni* correspondingly. The monthly activity of *P. papatasi* in indoor resting places started to appear in late April and ended in early October. This species had two peaks in indoors, one in early June and another in late August. The monthly activity of these sand flies in rodent burrows began in early May and ended in mid October. This species had two peak activities in outdoors, one in early June and the second in early September (Fig 1).

The sex ratio i.e. number of males/100 females of *P. papatasi* was 241.5 and 307.3 in indoors and rodent burrows respectively. Fifty six sand flies (3 *P. papatasi*, 48 *S. sintoni*, 3 *S. dentata* and 2 *S. clydei*) were collected in the vicinity of rodent burrows and dissected in September 2000 and 2001. Natural leptomonad infection was found only in 1/48 (2.08%) of *S. sintoni* but parasite characterization was not carried out (Table 2).

**Table 1:** The prevalence of ulcers and scars by age group and sex in Hossein-abad Mishmast village, Ghanavat rural district, Qom county, central Iran, Dec 1999

Age group(years)	Female				Male				Both sexes						
	No. of Observed	Scars		Acute lesions		No. of Observed	Scars		Acute lesions		No. of Observed	Scars		Acute lesions	
		No.	%	No.	%		No.	%	No.	%		No.	%	No.	%
1-4	41	3	7.32	3	7.32	42	3	7.14	1	2.38	83	6	7.23	4	4.82
5-9	59	10	16.95	3	7.08	63	8	12.70	5	7.94	122	18	14.75	8	6.56
10-14	84	10	11.90	6	7.14	95	12	12.00	5	5.26	179	22	12.29	11	6.15
15-19	76	9	11.84	4	5.26	79	8	12.63	3	3.80	155	17	10.97	7	4.52
20-24	66	8	12.12	3	4.55	75	5	10.13	4	5.33	141	13	9.22	7	4.96
>25	178	29	16.29	6	3.37	211	28	6.66	12	5.69	389	57	14.65	18	4.63
Total	504	12.44	13.69	25	4.96	565	64	11.33	30	5.31	1069	133	12.44	55	5.14

**Table 2:** Natural leptomonad infection in sandflies collected from rodent burrows, Ghanavat rural district, Qom county, central Iran, Sep 2000 and 2001

Species	No. of sandflies dissected	Stages in the gonothrophic cycle				Age group			No. of sandflies with leptomonads				% Infected	
		Unfed	Blood fed	Semigravid	Gravid	Nuliparous	Parous	Age not known	Total	Gut	Oesophagus	Head		
<i>P.papatasi</i>	3	1	0	2	0	0	3	0	0	0	0	0	0	0
<i>S. sintoni</i>	48	7	15	23	3	9	34	5	1	1	0	0	0	2.08
<i>S. dentata</i>	3	0	2	0	0	2	1	0	0	0	0	0	0	0
<i>S. clydei</i>	2	0	0	0	2	0	2	0	0	0	0	0	0	0

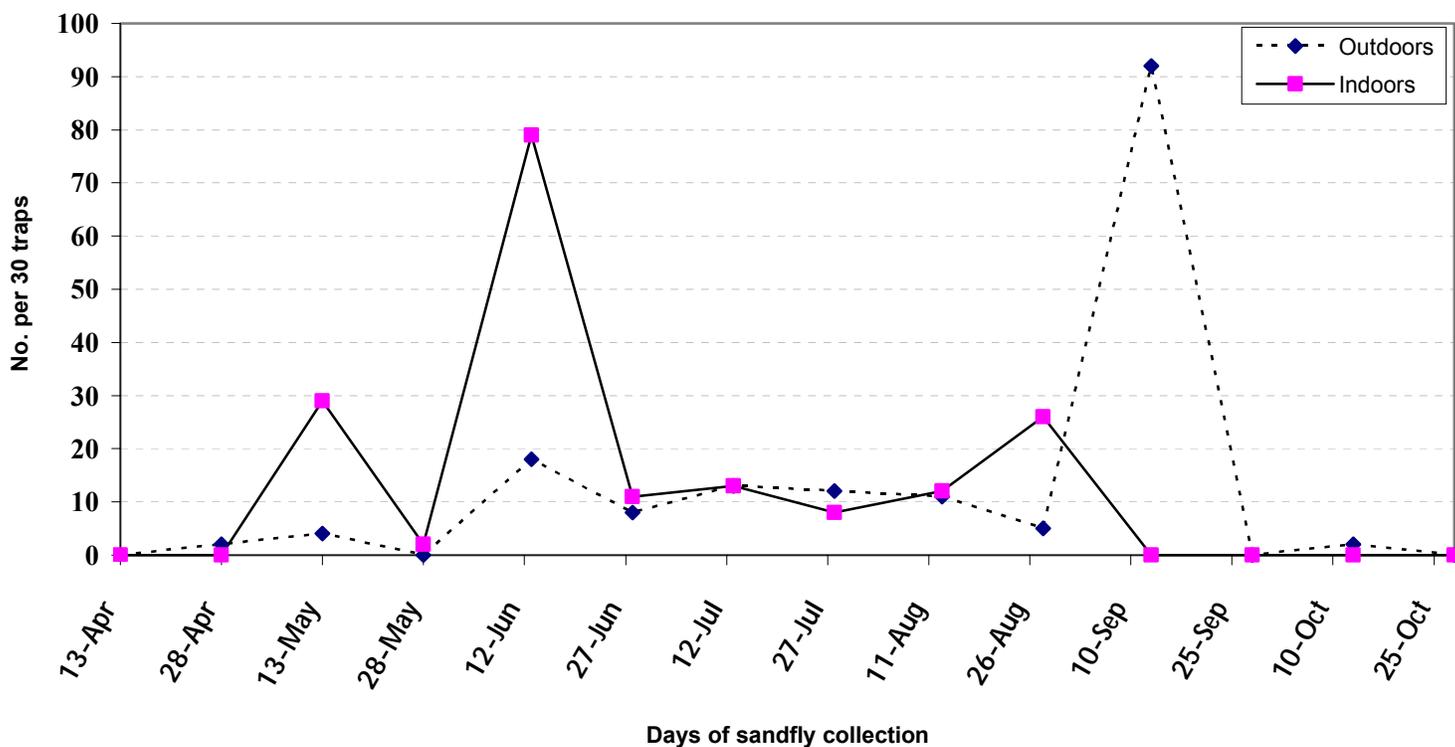


Fig.1. Monthly density of *P.papatasi* in Ghanavat rural district, Qom county, central Iran, 2000.

## Discussion

This is the first time that an outbreak of ZCL due to *L.major* has been identified in Ghanavat rural district, Qom province, central Iran. The same species of *Leishmania* parasite has been isolated from *P.papatasi*, *P.caucasicus*, *Rhombomys opimus*, *M.libycus*, *Tatera indica* and human in other parts of the country (1,6- 16). In the population survey, the ulcers were observed among all age groups. The  $\chi^2$  test showed non-significant differences in the prevalence of the ulcers above and under of ten years old ( $\chi^2= 0.26$ ,  $df=1$ ,  $P=0.609$ ). It can be concluded that an epidemic of ZCL was occurred during the study in the district. The  $\chi^2$  test also showed non-significant differences by sex ( $\chi^2=0.07$ ,  $df=1$ ,  $P=0.796$ ). Because of the high proportion of *M.libycus* (66.7%) in the Ghanavat rural district, it seems this species acts as the reservoir host in the focus. In some parts of Badrood rural district, Isfahan province, the main reservoir host is *M.libycus* (1). Natural leishmanial infection of this species

was also recorded around Isfahan, some parts of Khuzestan, Ardestan, Yazd and also Arsanjan (7, 9, 12, 13, 15). Our entomological survey showed that *P.papatasi* (81.53%) and *S.sintoni* (95.22%) were dominant species in indoor and outdoor resting places respectively. *Phlebotomus papatasi* is peridomestic in indoors. With regard to the existence of *P.papatasi* as peridomestic species in indoors and based on the isolation and characterization of *L.major* from this species in some other foci of ZCL in central Iran (11, 14) it seems that this species has the main role to transmit the parasite to man in the study area. We are dealing with ZCL in the Ghanavat rural district with an outbreak in 1999. *Leishmania major* is the agent and *M.libycus* and *P.papatasi* are as probable reservoir and vector respectively. The occurrence of this outbreak of ZCL in this district seems to be the result of construction of buildings near colonies of rodent burrows and also traveling to other ZCL foci of Iran. Following the experience gained from a

research project on ZCL control in Badrood rural district, central Iran (17) rodent control operations by using 2.5% zinc phosphide poisoned bait in a radius of 500 m from human dwellings is suggested for the control of the disease before the beginning of the active season of sand flies. Human individual protection, health education, active case detection and treatment are also recommended.

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