

Original Article**Emergence of Cutaneous Leishmaniasis due to *Leishmania major* in a New Focus of Southern Iran**AA Akhavan^{1, *}, MR Yaghoobi-Ershadi¹, F Hasibi¹, R Jafari², H Abdoli³, MH Arandian³, H Soleimani², AR Zahraei-Ramazani³, M Mohebali⁴, H Hajjaran⁴¹Dept. of Medical Entomology and Vector Control, School of Public Health and institute of Public Health Research² Yazd Health Research and Training Center³ Isfahan Health Research and Training Center, Medical Sciences/University of Tehran, Tehran, Iran⁴Dept. of Medical Parasitology, School of Public Health and institute of Public Health Research

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ABSTRACT

Reports from the health center of Kerman Province, southern Iran showed an increasing of cutaneous leishmaniasis cases in Orzuieh Rural District, southwest of the province in 2003. The report encouraged the team to carry out an epidemiological survey in the district during 2003-2004. The objectives were to determine the ecology of sand flies, potential reservoir hosts and human infection. A total of 1075 sand flies were collected by sticky traps and 7 species of sand flies were identified. They comprised 3 species of the genus *Phlebotomus* (*P. papatasi*, *P. mongolensis* and *P. bergeroti*) and 4 species of the genus *Sergentomyia* (*S. sintoni*, *S. clydei*, *S. tiberiadis* and *S. baghdadis*). *P. papatasi* was the predominant species of the genus *Phlebotomus* in indoors (90.3%) and outdoors (50.2%). Susceptibility tests on *P. papatasi* with DDT 4%. Showed that the species was susceptible to this insecticide. A total of 13 rodents consist of *Tatera indica* (76.9%) and *Nesokia indica* (23.1%) were collected. A study of prevalence among 2441 inhabitants in four villages showed a rate of 1.1% for active lesions and 10.4% for scars during November- December 2003. In a separate study examination of 1662 school children aged 6-12 years old showed 1.14% for ulcers and 14.7% for scars at the same time. The *Leishmania* parasites were isolated from man and characterized as *Leishmania major* using RAPD-PCR method. It seems that cutaneous leishmaniasis due to *L. major* (CLM) has been prevailed in the district.

Keywords: Sand flies, Epidemiology, *Leishmania major*, Leishmaniasis, Vector, Iran**INTRODUCTION**

Cutaneous leishmaniasis due to *Leishmania major* (CLM) is still a great and increasing public health problem in many rural areas of 15 out of 30 provinces of Iran (Yaghoobi-Ershadi, et al. 2005). At a particular geographical location where the disease has been sporadic in the past, it has grown to epidemic portions. It has been spread into sites where it did not previously exist (Yaghoobi-Ershadi et al. 2001a). Cutaneous leishmaniasis due to *L. tropica* (CLT) and also sporadic cases of CLM and visceral leishma-

niasis (VL) were reported from Kerman Province during the last 3 decades (Institute of Public Health Research, unpublished data). Recently a new focus of cutaneous leishmaniasis (CL) has been found in some villages of Orzuieh Rural District, southwest of Kerman Province, southern Iran. In 2002, a total of 100 cases were officially reported by passive case detection from Baft County and most of them were from Orzoieh Rural District, Kerman Province (Kerman Health Center, Unpublished data). Although CL is a notifiable disease in Iran but the real figures seem to be 4-5 folds. The region is

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the main pivot of agriculture of Kerman and it is famous as the granary of the province. This attracts many non-immune workers and they may be exposed to bites of infected sand flies during the active season.

However the epidemiological aspects of CL in this district had not been examined so we carried out ecological and epidemiological investigations for the first time in this focus for the implementation of future control measures.

The objectives of this study were to determine the prevalence of the disease, to study the reservoirs, ecology of sand flies, the vectors and also the nature of the parasite in this new focus.

MATERIALS AND METHODS

Study area

Field studies were conducted over a period of 12 months from August 2003 to September 2004 in four villages, Dowlatabad, Soltanabad, Shahmaran and Vakilabad in Orzuieh Rural District, Baft County, Kerman Province (56.24-56.38° E, 29.13-29.15° N), southern Iran. The choice of these villages was based on the increased number of the reported cases of CL by passive case detection in 2002 (Kerman Health Center, unpublished data). The area has a desert climate, altitude between 1050-1095, hot in summer and quite cold in winter. In 2003, the maximum and minimum mean monthly temperatures were 33.1 °C and -0.4 °C in July and February, respectively. The total annual rainfall was 309 mm with a minimum of 3 mm in July and maximum of 120.9 mm in April. The minimum mean monthly relative humidity was 26% (June) and the maximum was 56% (January). Wheat, Indian corn, cotton, sesame plants, alfalfa, onion, sunflowers, watermelon, date, citrus fruits, pistachio, and pomegranate are the common crops in the region. The Orzuieh River runs among the infected villages.

Population studies

One hundred and sixteen households of each village whose buildings were located near each other were examined in December 2003,

and corresponding forms were completed for each household during a house-to-house visit. The presence or absence of scars or ulcers of CL were indicated on the forms. We also examined and questioned all the students of the school children in the surveyed area simultaneously. Each individual was examined for scar(s) or ulcer(s) and the date and place of acquiring the disease, age, sex, number of ulcer(s) or scar(s), site of ulcer(s) or scar(s) were recorded.

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 using SPSS 11.5, was used to determine statistically significant differences in disease prevalence between females and males and among different age groups in the community. The graphs were also prepared by Excel®.

Isolation of the parasites from patients

Samples from two patients, with no traveling history to the other foci of CLM, were taken from ulcers and inoculated subcutaneously at the base of the tail of 6 BALB/c 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 at 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 (Mohebbi et al. 2002, Motazedian et al. 2002).

Collection and examination of rodents

Small mammals were caught by 20 live traps baited with cucumber and tomato, 16 times during different seasons in 2003 and 2004. In the laboratory, 4 impression smears were prepared from the ears of each mammal (Edrissian

et al. 1982), fixed in methanol and stained by the Giemsa method and examined carefully under the light microscope.

Collection of sand flies

Sand flies were collected from indoor (bed rooms, warehouse, toilets, and hall) and outdoor (rodent burrows) fixed places, using 30 sticky traps in each (castor oil coated white paper 20 x 32 cm) from sunset to sunrise and also by aspirator from indoors (6.00 to 10.00 AM) four times in June, August, September, and October in 2004. For species identification, sand flies were mounted in Puri's medium (Smart et al. 1965) and identified after 24 h using the morphological characters (Theodor and Mesghali 1964). Then they were counted and segregated by sex. In order to determine natural promastigote 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 in August and September 2003-2004. The graphs were prepared by Excel®.

Susceptibility test

The susceptibility status of *Phlebotomus papatasi* to DDT was studied in the field surveys during September 2004 following the WHO standard method (WHO 1981) and using impregnated papers supplied by WHO.

RESULTS

A study of prevalence among 2441 inhabitants from 495 households in four villages showed a rate of 1.11% for active lesions during November- December 2003 (Table 1). The most highly infected age group was 0-4 yr with a rate of 3.35%. Children under 10 yr of age had a rate of 2.02% for active lesions. The rate was 0.93% for those more than 10 yr old. Chi-square analysis indicates that there is significant differences in number of individuals with active lesions among different age groups ($X^2= 15.858$, $df= 5$, $P< 0.007$). The prevalence of scars was calculated to be 10.37% (Table 1). The scar rate

was 6.55% for individuals under 10 yr of age and 11.11% for those over 10 yr of age. The proportion of males and females were 50.5% and 49.5% respectively. Significant differences in number of individuals with scars were also observed among different age groups ($X^2= 40.307$, $df= 5$, $P< 0.0001$). At the same time, altogether, 14 primary schools with 1662 students (885 boys and 777 girls) from 6 to 12 yr of age visited. The overall scar rate was 14.68% and the prevalence of active lesions was 1.14% (Table 2).

Most of the patients had one active lesion and most of them were observed on the hands, legs and face. Microscopic slides prepared by scraping the edges of the lesions of all patients (inhabitants of households and students of primary schools), contained parasites presumed to be *Leishmania major* based on existence of a large vacuole in the cytoplasm. Treatment was provided for 46 subjects with a parasitological diagnosis of leishmaniasis. Examination of two isolates from human indigenous cases identified them as *L. major*.

A total of 13 rodents consist of *T. indica* (76.9%) and *N. indica* (23.1%) were collected and examined for leishmanial infection during August- September 2003- 2004 but all were found to be negative.

During June-October 2004 a total of 1075 sand flies (310 from indoors and 765 from outdoor resting places) were collected and identified. The following 7 species were found in bedrooms, storerooms and toilets: *P. papatasi* (90.3%), *P. mongolensis* (0.3%), *P. bergeroti* (0.7%), *Sergentomyia sintoni* (5.1%), *S. clydei* (0.7%), *S. tiberiadis* (0.7%) and *S. baghdadis* (2.2%) (Fig.1). In rodent burrows: *P. papatasi* (50.2%), *P. mongolensis* (0.1%), *S. sintoni* (35%), *S. clydei* (11.5%), *S. tiberiadis* (2.8%) and *S. baghdadis* (0.4%) were collected (fig.2). Nine *P. papatasi* and 1 *S. sintoni* in the vicinity of rodent burrows and 45 *P. papatasi* from indoors were collected and dissected in September 2003. None of them were found to be infected with promastigotes. A total of 92 *P. pa-*

papatasi and 2 *S. sintoni* were collected from the vicinity of rodent burrows again in the area and dissected in September 2004. None of them appeared to be infected.

The sex ratio, i.e. number of males per 100 females, of *P. papatasi* collected by sticky traps, in indoors and outdoors was calculated to be 156.9 and 193.13, respectively. In case of *S. sin-*

toni it was found 45.4 and 57.7 in indoors and outdoors respectively.

A total of 107 fed *P. papatasi* were collected by aspirator from indoors in four villages in first half of September 2004. The mortality rate for DDT 4.0% after 6 0.00 min exposure time followed by 24 h recovery time was 100%. It is concluded that *P. papatasi* strain of Orzuieh is susceptible to DDT.

Table 1. The prevalence of cutaneous leishmaniasis by age among 495 families (both sexes) in the study area, Nov- Dec 2003

Age group (yr)	No. observed	No. with scars	%	No. with active lesions	%
0-4	179	4	2.24	6	3.35
5-9	218	22	10.09	2	0.92
10-14	379	64	16.89	1	0.26
15-19	444	53	11.94	2	0.45
20-24	273	36	13.19	1	0.37
25+	948	74	7.81	15	1.58
All groups	2441	253	10.37	27	1.11

Table 2. The prevalence of active lesions and scar rate by age among the students (both sexes) of primary schools in the study area, Nov- Dec 2003

Age (yr)	No. observed	No. with scars	%	No. with active lesions	%
6	41	0	0	0	0
7	284	21	7.39	3	1.06
8	300	43	14.33	1	0.33
9	329	47	14.29	3	0.91
10	348	51	14.66	5	1.44
11	356	79	22.19	6	1.69
12	4	3	75	1	25
Total	1662	244	14.68	19	1.14

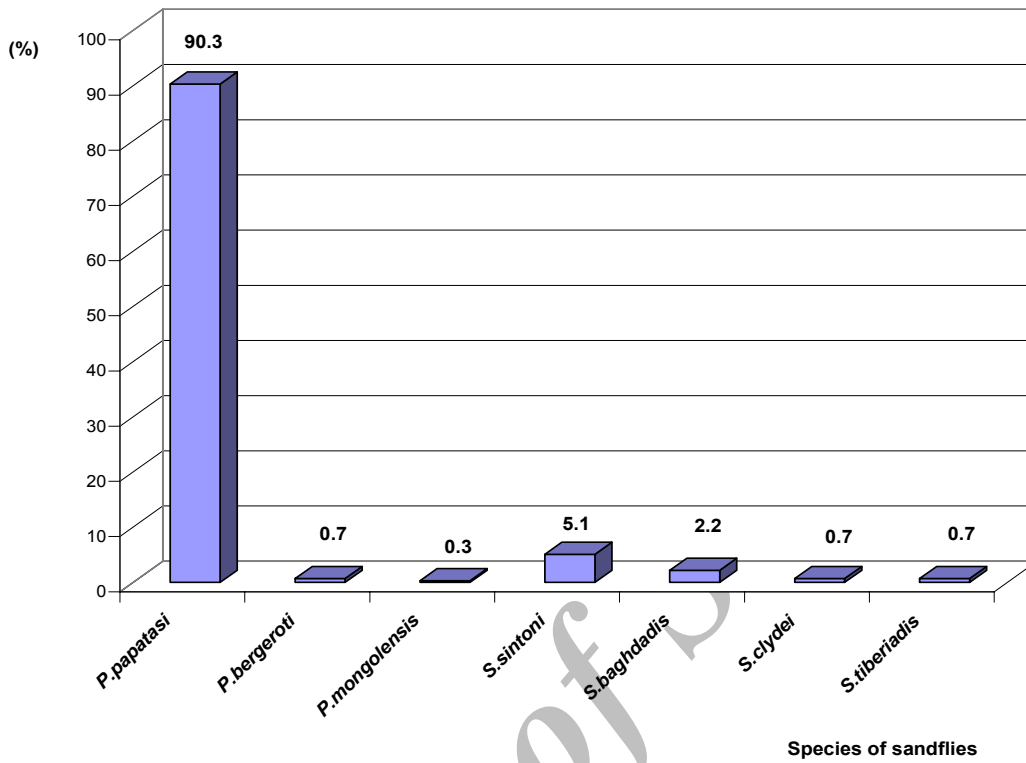


Fig. 1. The fauna of sand flies from indoor resting places, Orzuieh Rural District, southwest of Kerman Province, southern Iran, 2003 and 2004.

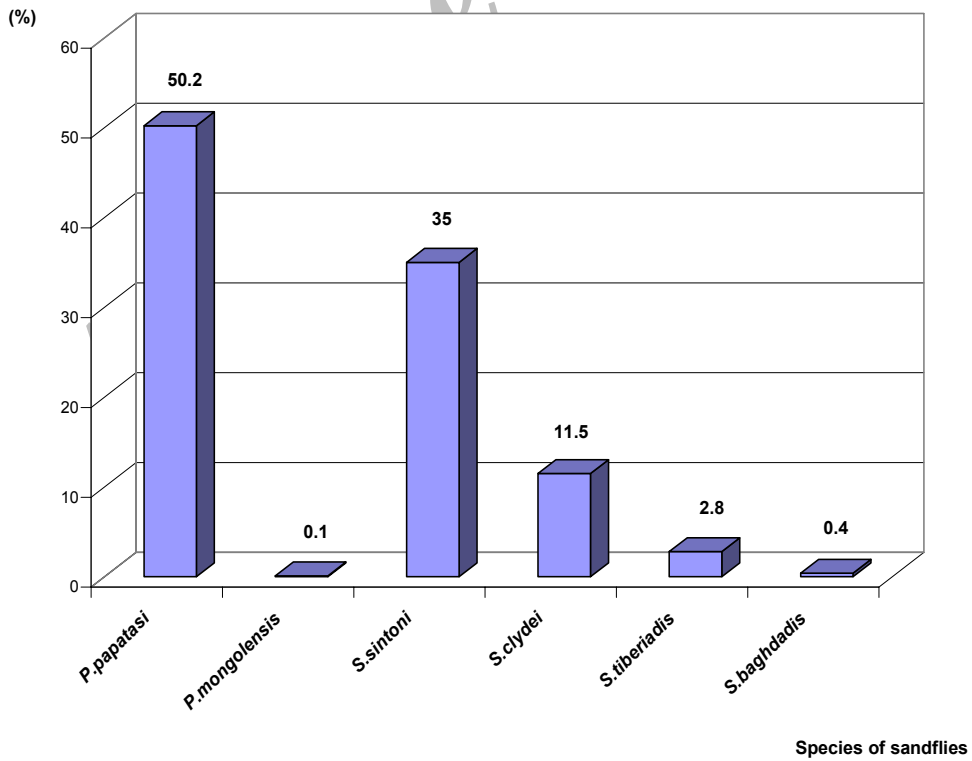


Fig. 2. The fauna of sand flies from rodent burrows, Orzuieh Rural District, southwest of Kerman Province, southern Iran, 2003 and 2004.

DISCUSSION

This study was carried out on some epidemiological aspects of CL in the study area for the first time in Iran. *L. major* isolated and characterized from the lesions of the patients with no history of traveling to the other CLM foci of Iran.

The same species of *Leishmania* parasite has been isolated from *P. papatasi*, *P. caucasicus*, *Rhombomys opimus*, *Meriones libycus*, *Tatera indica* and human in other parts of the country (Akhavan et al. 1998 and 2003, Javadian et al. 1998, Rassi et al. 2001, Yaghoobi-Ershadi et al. 1994, 1995, 1996a, 1996b, 2001a, 2001b, 2001c, 2002). *Tatera indica* is the common gerbil in Orzoieh Rural District. Because of the high proportion of *T. indica* (76.9%) in the district, it seems this species may play a role as the reservoir host in the focus. Natural *Leishmanial* infection of this species was also recorded from Khuzistan and Ilam Provinces of Iran (Javadian et al. 1988, 1998). This is the first study that reports 7 species of sand flies from Baft County and *P. bergeroti*, *P. mongolensis* and *S. clydei* are also new records for Kerman Province. *P. papatasi* was the predominant species of the genus *Phlebotomus* in indoors (90.3%) and outdoors (50.2%). Regarding 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 CLM in central Iran (Yaghoobi-Ershadi 1995, 2001, 2005a) and also from patients and *M. libycus* in Fars Province nearby the study area (Rassi et al., 2001) it seems that probably this species acts as the vector to man and also among rodents. Over the last decade, cases of CLM have been reported from the west and southwest of the central desert and even from the south of the country (Neiriz, Estahban, Lar, Darab, Jahrom, Kharam, Sarvestan, Arsanjan, Hadjiabad and Kahurestan (Yaghoobi-Ershadi et al. 2005b). It seems that Orzoieh is also extension of these foci. Cutaneous leishmaniasis due to *L. major* (CLM) has been prevailed in the district. *P. papatasi* and *T. indica* are the probable vector and reservoir respectively.

The occurrence of this outbreak of CLM in the study villages seems to be the result of development of agro-industrial activities, instruction of buildings nearby colonies of gerbils and their venturing inside houses, sleeping of inhabitants in courtyards at night during active season of sand flies.

Following the experience gained from a research project on CLM control, central Iran (Yaghoobi-Ershadi et al. 2005b), destroying of gerbils with zinc phosphide mixed with wheat grains and vegetable oil (2.5%) within a radius of 500 meters of houses once a month during May, June, July and September in the first year and once every two years in the coming years is suggested for the control of the disease.

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