

Original Article**Some Ecological Aspects of Phlebotomine Sand Flies in an Endemic Focus of Cutaneous Leishmaniasis in Iran**

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Abstract

Following annual report of new cases of cutaneous leishmaniasis from different areas of Kuhpayeh district, Esfahan County, an investigation was carried out on some ecological aspects of Phlebotomine sand flies during 2000-2002. Sand flies were collected biweekly from outdoor and indoor resting places with the aid of 30 sticky traps from the beginning to the end of the active season. Female sand flies from rodent burrows were dissected and examined for the presence of promastigote infection. Blood meals of engorged sand flies were identified by ELISA method. Totally, 4993 sand flies were collected and identified. The following species were found indoors: *P. papatasi*, *P. sergenti*, *P. caucasicus*, *P. mongolensis*, *P. alexandri*, *P. ansarii*, *P. major*, *P. kandelakii*, *S. sintoni*, and the subsequent species were found outdoors: *P. papatasi*, *P. sergenti*, *P. caucasicus*, *P. mongolensis*, *P. ansarii*, *P. major*, *S. sintoni*, *S. dentata*, *S. pawlowskyi*. The sand flies active season began from May till October in this region. Natural promastigote infections observed in 1.06% of *P. papatasi* and also in one out of four of *P. caucasicus*. The human and rodent blood indices in *P. papatasi* were 61.9% and 20.69%, respectively. It seems that *P. papatasi* is the probable vector among rodents and also transmit *Leishmania major*, the causative agent of zoonotic cutaneous leishmaniasis to man, and *P. caucasicus* transmit the agent of the disease among rodents.

Keywords: Ecology, Leishmaniasis, Phlebotomine sand flies, Iran

Introduction

Phlebotomine sand flies are the vectors of leishmaniasis and sand fly fever in Iran (Nadim and Seyedi-Rashti 1971, Tesh et al. 1976, Yaghoobi-Ershadi et al. 2005). Cutaneous leishmaniasis due to *L. major* (CLM) is a great health problem in the country and prevails in rural districts of 15 out of 30 provinces in Iran (Akhavan et al. 2007). Kuhpayeh district is one of the endemic foci of cutaneous leishmaniasis

(CL), 30 km east of Esfahan city, central Iran. The incidence of the disease was reported 4.1/1000 in 1996 (Esfahan Health center, unpublished data). The infected district is located close to the Esfahan City, one of the most important historical and industrial cities of Iran and lots of people from various parts of Iran and also other countries visit it during the active seasons of sand flies.

The main vector to human in Iran is *Phlebotomus (Phlebotomus) papatasi* Scopoli

(Diptera: Psychodidae), and the other vectors among rodents in the rural areas are *P. (Paraphlebotomus) caucasicus* Marzinowsky, *P. (Pa.) mongolensis* Sinton, *P. (Pa.) andrejevi* Shakirzyanova, *P. (Pa.) alexandri* Sinton, and *P. (Synphlebotomus) ansarii* Lewis (Nadim et al. 1968, 1994, Javadian et al. 1977, Yaghoobi-Ershadi et al. 1994, 1995b, Rassi et al. 2007). *L. major* has been isolated from *P. papatasi* and *P. caucasicus* in some CLM foci of Iran (Yaghoobi-Ershadi et al. 1994, 1995 b, 2001 a, 2001 b, 2005, Rassi et al. 2007).

The ecological aspects of the CL vectors have not been examined in Kuhpayeh district yet. Therefore some ecological aspects of sand flies were studied for the first time in this district for implementation of future control measures. The objectives of the present study were to determine the sand flies fauna, species composition, population density, number of generation and also leishmanial infection rate of sand flies in the area.

Materials and Methods

The investigation was conducted during 2000-2002 in 4 areas of Kuhpayeh district, Esfahan Province, central Iran. The city of Kuhpayeh is the center of the district (32° 42'N, 52° 26'E) east of Esfahan City. The district has different plain and mountainous climate at various altitudes, about 1550 to 1780 m.

The maximum and minimum mean monthly temperatures were 37.9 °C and -5 °C in July and February, respectively. The total annual rainfall was 70 mm with a minimum of 0.4 mm in April and maximum of 29.9 mm in December during rainy months. The minimum mean monthly relative humidity was %26 (July) and the maximum was %57.5 (December).

Sand flies were collected biweekly from indoor (bedrooms, hall, toilets and stables) and outdoor (rodent burrows, mountain and rock rifts) fixed places in 9 parts of the areas for detecting monthly variation and also 11

points for detecting the fauna of sand flies, using 30 sticky traps (castor oil coated white paper 20×32 cm) from the beginning (April) through the end (October) of the active season. For species identification, sand flies were mounted in Puri's medium (Smart et al. 1965) and identified after 24 h using the keys of Theodor and Mesghali (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, semi gravid 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. To determine the host preference of *P. papatasi*, the sand flies collected by aspirating tube and sticky paper traps from human dwelling, rodent burrows and stables. The sand flies were dissected freshly and blood meals smeared onto circles of Whatman No. 1 filter paper. They were interleaved with non-absorbent onionskin paper and sent with necessary information to the Department of Parasitology in the Pasteur Institute of Iran for enzyme linked immunosorbent assay (ELISA) testing (Edrissian et al. 1985). Because of small quantity of blood meals, each one was tested against only one antiserum.

Results

A total of 4993 adult sand flies (4294 from outdoors and 699 from indoors) were collected during May-October 2000 and 11 species of sand flies (8 species of the genus *Phlebotomus* and 3 species of the genus *Sergentomyia*) were identified. The following species were found in indoors: *P. papatasi* (27.43%), *P. sergenti* (52.04%), *P. caucasicus* and *P. mongolensis* (4.39%), *P. alexandri* (0.31%), *P. ansarii* (0.78), *P. major* (2.04%), *P. kandelakii* (1.72%), *S. sintoni* (11.29%) and the subsequent species were found in outdoors: *P. papatasi* (35.04%), *P. sergenti* (2.03%), *P. caucasicus* and *P. mon-*

golensis (3.65%), *P. ansarii* (1.15%), *P. major* (0.05%), *S. sintoni* (57.73%), *S. dentata* (0.28%), *S. pawlowskyi* (0.07%).

Six species of Phlebotomine sand flies were collected from plain areas and 11 species were found in mountain areas (Table 1). The most common sand flies in plain areas were *P. papatasi* (37.39%) and *S. sintoni* (58.23%) and the most abundant species in mountain areas was *P. sergenti* (71.3%). These species were collected in all of the months during the active season. The monthly activity of *P. papatasi* and *P. sergenti* began in late April and ended in late September with two peaks of activ-

ity (Fig. 1 and 2). No sand fly was found in the area during November to March due to cold weather. The sex ratio i.e. number of males/100 females of *P. papatasi*, *P. sergenti* and *S. sintoni* was 110.53, 357.89 and 18.64 in outdoors and also 182.26, 1085.71 and 41.18 in indoors, respectively (Table 2). Natural promastigote infections observed in 1.06% of *P. papatasi* and also in one out of four of *P. caucasicus* but attempt to isolate the parasite failed (Table 3). ELISA testing of 52 *P. papatasi* showed that the human and rodent blood indexes in *P. papatasi* were 61.9% and 20.69%, respectively.

Table 1. The diversity of sand flies in plain and mountain areas, Kuhpayeh district, Esfahan Province, central Iran, 2000.

| Species Area | <i>P. papatasi</i> | <i>P. sergenti</i> | <i>P. mongolensis</i> | <i>P. caucasicus</i> | <i>P. alexandri</i> | <i>P. ansarii</i> | <i>P. kandelakii</i> | <i>P. major</i> | <i>S. sintoni</i> | <i>S. dentata</i> | <i>P. pawlowskyi</i> |
|----------------|--------------------|--------------------|-----------------------|----------------------|---------------------|-------------------|----------------------|-----------------|-------------------|-------------------|----------------------|
| Plain areas | + | + | + | + | | + | | | + | | |
| Mountain areas | + | + | + | + | + | + | + | + | + | + | + |

Table 2. The sex ratio of sand flies collected by sticky paper method from indoor and outdoor resting places, Kuhpayeh district, Esfahan Province, central Iran, 2000

| Resting places | Indoors | | | | | | Outdoors | | | | | |
|--------------------|---------|---------|------|---------|-------|-----------|----------|---------|------|---------|-------|-----------|
| | Female | | Male | | Total | Sex Ratio | Female | | Male | | Total | Sex Ratio |
| | No | Percent | No | Percent | | | No | Percent | No | Percent | | |
| <i>P. papatasi</i> | 62 | 35.43 | 113 | 64.57 | 175 | 182.26 | 712 | 47.5 | 787 | 52.5 | 1499 | 110.53 |
| <i>P. sergenti</i> | 28 | 8.43 | 304 | 91.57 | 332 | 1085.71 | 19 | 21.84 | 68 | 78.16 | 87 | 357.89 |
| <i>S. sintoni</i> | 51 | 70.83 | 21 | 29.17 | 72 | 41.18 | 2082 | 84.29 | 388 | 15.71 | 2470 | 18.64 |

Table 3. Natural leptomonad infection rate of sand flies from rodent burrows, Kuhpayeh district, Esfahan Province, central Iran 2000-2002

| Species | No. Dissected | Age Group | | | No. sand flies with leptomonads | | | | Infected% |
|----------------------|---------------|-----------|----|----|---------------------------------|---|---|---|-----------|
| | | N | P | ? | T | G | E | H | |
| | | | | | | | | | |
| <i>P. papatasi</i> | 94 | 13 | 70 | 11 | 1 | 1 | - | - | 1.06 |
| <i>P. caucasicus</i> | 4 | 3 | 1 | - | 1 | 1 | - | - | 25 |
| <i>P. ansari</i> | 1 | - | - | - | - | - | - | - | - |

N: nulliparous, P: parous,?: Age not known, T: total, G: gut, H: head, E: esophagus

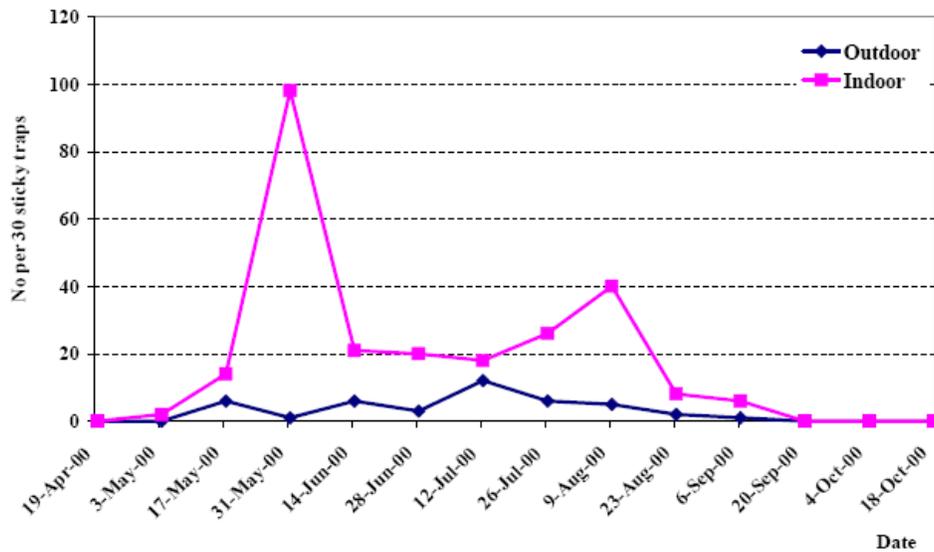


Fig. 1. The monthly variation of *P. sergenti* from indoor and outdoor resting places, Jebel rural area, Kuhpayeh district, Esfahan Province, central Iran, 2000

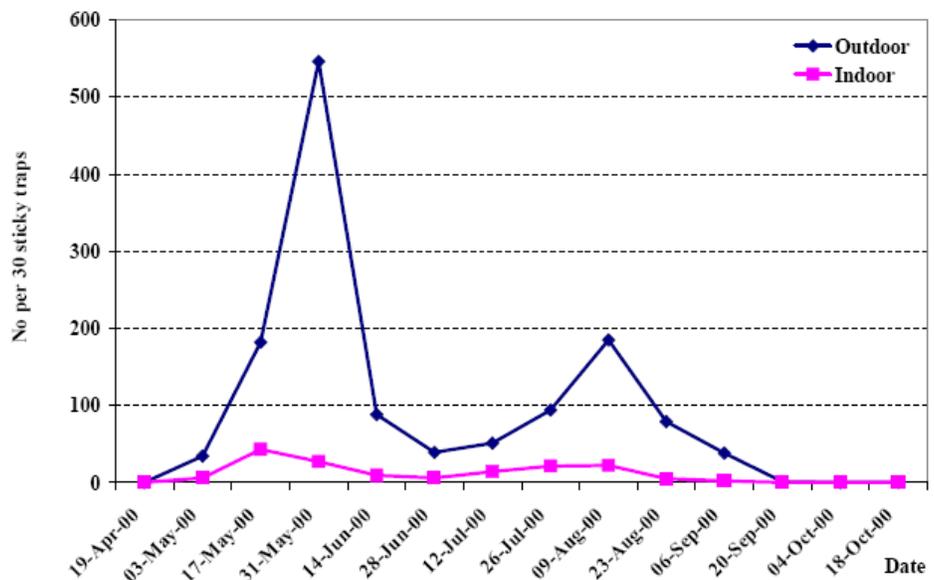


Fig. 2. The monthly variation of *P. papatasi* from indoor and outdoor resting places, Sistan rural area, Kuhpayeh district, Esfahan Province, central Iran, 2000

Discussion

This is the first report of some ecological aspects of sand flies in Kuhpayeh rural district, Esfahan Province, central Iran. Six species of Phlebotomines were identified in plain areas and 11 species in mountain areas. The diversity of sand flies in mountain areas was more than plains. *P. papatasi* and *P. sergenti*

are the dominant sand flies of the genus *Phlebotomus* with two generations in the plain areas. *P. papatasi* and *P. sergenti* are peridomestic species. Blood meal analysis shows that the anthropophilic index of *P. papatasi* (61.9%) is high as has been found by some other investigators (Natima et al. 1991). The anthropophilic index of this species has been reported to be 29.6-44.6% and 31.2% in

Borkhar and Badrood districts of Esfahan Province, respectively (Yaghoobi-Ershadi et al. 1995a, 2005). Natural promastigote infection rate of *P. papatasi* from rodent burrows was 1.06%. Natural infection of this species was also recorded from Badrood (6.7-22%), Nikabad (4.76%), Sabzevar (4.5%), Lotfabad (5.8%) and Esfarayen (2.2%) (Mesghali et al. 1967, Javadian et al. 1976, Yaghoobi-Ershadi et al. 2001a, 2001b, 2003, 2005). *L. major* has been isolated from *P. papatasi* and *P. caucasicus* in some CLM foci of Iran (Yaghoobi-Ershadi et al. 1994, 1995b, 2001a, 2001b, 2005, Rassi et al. 2007). Naturally promastigote infection of *P. caucasicus* has been reported from a focus of zoonotic visceral leishmaniasis (ZVL) in Iran (Rassi et al. 2004). *L. major* has been isolated from gerbils and also human in some CLM foci of Iran (Yaghoobi-Ershadi et al. 1996, Akhavan et al. 1998, Rassi et al. 2007).

The close contact between vectors and reservoir hosts have created a very efficient cycle for transmitting the disease among rodents and also transmission the disease to people living in the villages located near rodent burrows.

Regarding the results of the present study and based on the isolation and characterization of *L. major* from *P. papatasi* and *P. caucasicus* in neighboring focus of the disease in Esfahan Province (Yaghoobi-Ershadi et al. 1994, 1995b) it seems that *P. papatasi* has the main role to transmit the parasite among rodents and also to man and *P. caucasicus* transmit the agent of the disease among rodents. To confirm the focus of CLM in this district, isolation and characterization of *L. major* from *P. papatasi*, great gerbil and also human is recommended.

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