

Bionomics of *Phlebotomus papatasi* (Diptera: Psychodidae) in an endemic focus of zoonotic cutaneous leishmaniasis in central Iran

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ABSTRACT: Following an epidemiological survey of zoonotic cutaneous leishmaniasis (ZCL) in several villages of Badrood, a rural district north of the city of Natanz, central Iran, *Phlebotomus (Phlebotomus) papatasi* Scopoli were found to be naturally infected with *Leishmania (Leishmania) major* zymodeme MON-26. Sand flies were collected and dissected biweekly from rodent burrows from May to October 2001. Leptomonad infection rates varied between 6.7% and 22.0%, being greatest in September, coinciding with peak activity of *P. papatasi*, two-three months before the highest incidence of ZCL human cases in November-December. The leptomonad infection rate was 1.1% of the 94 *P. papatasi* captured indoors. In ELISA testing of 520 *P. papatasi* blood meals during Sept. 2001 and Aug. 2002, the proportion giving positive reactions for human, sheep, cow, goat, rodent, and bird were 31.2%, 69.6%, 63%, 38.8%, 24.7%, and 21.8%, respectively. This report thus incriminates *P. papatasi* as the vector of zoonotic cutaneous leishmaniasis in this part of Iran. **Journal of Vector Ecology 30 (1): 115-118. 2005.**

Keyword Index: Ecology, leishmaniasis, *Phlebotomus papatasi*, infection rate, host preference, Iran.

INTRODUCTION

Zoonotic cutaneous leishmaniasis (ZCL) is endemic in 14 of the 28 provinces of Iran. In recent years a new focus of ZCL was found in villages of Badrood rural district, Natanz county among the foothills of the Karkas Mountains in central Iran. A study of 3,119 inhabitants showed a prevalence rate of 27% for active lesions and 2.3% for scars in three villages in 1995 (Akhavan et al. 1998). *Rhombomys opimus* (the Great Gerbil) and *Meriones libycus* (the Libyan Jird) are the main reservoir hosts of ZCL in the district (Yaghoobi-Ershadi et al. 1996).

The main vector to humans is *Phlebotomus (Phlebotomus) papatasi* Scopoli (Diptera: Psychodidae), and other vectors among rodents in rural areas are *Phlebotomus (Paraphlebotomus) alexandri* Sinton, *P. (Paraphlebotomus) andrejevi* Shakirzyanova, *P. (Pa.) caucasicus* Marzinowsky, *P. (Pa.) mongolensis* Sinton, and *P. (Synphlebotomus) ansarii* Lewis (Nadim et al. 1968, 1994, Javadian et al. 1977, Yaghoobi-Ershadi et al. 1994,1995). The following six species of sand flies were found in Badrood rural district: *P. papatasi*, *P. caucasicus*, *P. mongolensis*, *P. alexandri*, *P. sergenti*, and *S. sintoni*. *P. papatasi* is the most predominant sand fly in this region, constituting 94.1% and 61.4% of the total sand flies captured indoors and in rodent burrows respectively (Yaghoobi-Ershadi and Akhavan 1999).

Leishmania major zymodeme MON-26 has been isolated only from *P. papatasi* and *P. caucasicus* in Borkhar rural

district, north of the city of Isfahan (Yaghoobi-Ershadi et al. 1994,1995a), but nothing more is known of the identity of the parasites in vectors in the other foci of ZCL in our country. The present study investigated the potential role of *P. papatasi* in the epidemiology of ZCL in this focus. This paper reports the isolation and characterization of *L. major* (MON-26) from *P. papatasi*, its monthly variation and feeding preference in the ZCL focus of Badrood rural district for the first time in Iran.

MATERIALS AND METHODS

Study area

The investigation was carried out during 2001-2002 in three villages (Matinabad, Fami, and Abbasabad) in the rural district of Badrood (33°44' N, 52°2' E), 5-13 Km from the city of Badrood (Natanz county) Isfahan province, central Iran. Badrood is situated at an altitude of 1,056 m among the foothills of the Karkas mountains (altitude 3,898 m). The area has a desert climate, very hot in summer and quite cold in winter. In 2001, the maximum and minimum mean monthly temperatures were 42.1 °C and -3 °C in July and December, respectively. The total annual rainfall was 48 mm. The minimum monthly relative humidity was 19% (July) and the maximum was 64% (January).

Methods

Phlebotomine sand flies were collected biweekly from May to October 2001 from outdoor resting sites (gerbil and Jird burrows) and indoors using 30 sticky traps that were deployed overnight. The flies were also collected by aspirator and light traps from inside resting places. All fed gravid and semigravid females were dissected in a fresh drop of 0.9%w/v NaCl and examined microscopically for flagellate infection. When flagellates were seen, a few drops of saline were added to the preparation, which was then aspirated into a sterile syringe and injected subcutaneously into the bases of the tail of 2 BALB/c mice. Parasites were later isolated from infected mice and cultured in two to four tubes of Novy-Nicolle-MacNeal (NNN) medium plus Liver Infusion Tryptose (LIT) biphasic medium with penicillin (5×10^6 per ml).

Culture tubes were incubated at 20°C and subcultured every 15 d. All female sand flies were mounted in Puri's medium (Smart et al. 1965) and identified after 48 h by the morphology of the pharyngeal armature and spermatheca (Theodor and Mesghali 1964). The physiological age of each female was determined by the presence or absence of granules in the accessory glands (Foster et al. 1970).

Cultured promastigotes isolated from *P. (P.) papatasi* were sent by air to the World Health Organization Reference Center for the leishmaniasis, Faculty of Medicine, University of Montpellier, France (Professor J.P. Dedet and Dr. F. Protlong) for cryopreservation and isoenzyme characterization.

In order to study the host preference pattern of *P. papatasi*, sand flies were collected by sticky traps from human dwellings, rodent burrows, domestic bird nests, and stables in the village of Abbasabad (Badrood rural district) during September 2001 and August 2002. Each sand fly was dissected and blood meals smeared onto circles of Whatman No.1 filter paper (Theodor and Mesghali 1964). They were interleaved with non-absorbent onionskin paper and sent with the 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 the small quantity (0.3-0.5 mg) of blood ingested by sand flies, each blood meal was tested against only one antiserum.

RESULTS

From May until late October 2001, biweekly sticky trap collections in three villages (60 traps/village/month) yielded totals of 141 *P. papatasi*, two *P. caucasicus* and 17 *S. sintoni* from the vicinity of rodent burrows. Over 15% of *P. papatasi* and 6% of *S. sintoni* were infected with leptomonads. No leptomonads were found in *P. caucasicus*. Leptomonad infections in *P. papatasi* began to appear in mid-May peaked in the middle of September at a rate of 22% and fell to zero by the end of October. Leptomonads were also seen in the esophagus of 59% of the positive flies and in the head of 23% of the positive *P. papatasi* (Table 1). Out of 94 *P. papatasi* collected from indoor resting places during the first half of September 2001, only one specimen (1%) was infected.

The highest parous rate (97% *P. papatasi*) occurred in older populations towards the end of the transmission season in September. The highest rate of parasite infestation in *P. papatasi* (22%) was observed when the parous rate was high. Promastigotes from 13 heavily infected *P. papatasi* were injected subcutaneously in the tail bases of two BALB/c mice. Six of these 26 mice (23%) became infected and nodules and ulcers, containing numerous amastigotes developed at the site of inoculation 60-150 d after injection. Promastigotes grew well by 5 d after inoculation in eight of the 20 cultures inoculated with parasites from infected mice. The isolate from one fly was characterized as *L. major* zymodeme MON-26.

ELISA testing of 520 blood meals of *P. papatasi* showed that sheep and cow are preferred hosts of this species. The anthropophilic index was calculated to be 31.2 % (Table 2).

Table 1. Monthly variation of leptomonad infection rate in *P. papatasi* from rodent burrows, Badrood rural district, Isfahan province, Iran, 2001. All *P. papatasi* collected were dissected.

Month	No. Dissected	Age group			No. of sand flies with leptomonads					% Infected
		N	P	?	T	G	E	H		
May	14	3	10	1	1	1	1	0		7.1
June	1	0	0	1	0	0	0	0		-
July	15	2	12	1	1	1	0	0		6.7
August	29	5	23	1	2	2	1	1		6.9
September	59	0	57	2	13	13	6	3		22.0
October	23	0	22	1	5	5	5	1		21.7
Total	141	10	124	7	22	22	13	5		15.6

T, total; E, esophagus; G, gut; N, nulliparous.

H, head; P, parous; ?, age not known*.

* Some females that oviposited all of their eggs had accessory glands devoid of identifiable granules so recognizing parous sand flies from nuliparous ones was impossible.

Table 2. Host preference pattern of *P. papatasi* collected from Badrood rural district, Natanz county, Isfahan province, Iran. Sep. 2001 and Aug. 2002.

Engorgement source	Total No. of smears *	Positive rate (%)
Human	125	31.2
Sheep	79	69.6
Cow	81	63.0
Goat	80	38.8
Rodent	77	24.7
Bird	78	21.8

* *P. papatasi* were collected from human dwellings, stables, rodent burrows, and bird nests.

DISCUSSION

This is the first report of the isolation, characterization, and monthly variation of *L. major* MON-26 from *P. papatasi* in this new focus, which has been located nearby a holy place called Immamzadeh Agha-Ali-Abbas in Iran. Thousands of people visit this sacred place during the active season of sand flies and all are potentially exposed to the disease agent. *L. major* MON-26 is the agent of ZCL from the sub-Saharan Sahel to the near Middle East (Khiami et al. 1991). In 1995 we isolated MON-26 from *M. libycus* and humans in the study area (Akhavan et al. 1998, Yaghoobi-Ershadi et al. 1996) and also from *P. caucasicus* and *P. papatasi* in Borkhar, a rural district north of the city of Isfahan, about 200 km away from this new focus (Yaghoobi-Ershadi et al. 1994, 1995a).

Leishmanial infection rates of *P. papatasi* from rodent burrows of other ZCL foci in Iran (i.e. Abardezh, Ahwaz, Dezful, Isferayen, Lotfabad, Shush, Turkman-Sahra, and Isfahan) ranged from 0.2% to 10.9% during 1967-1991 (Yaghoobi-Ershadi et al. 1995, Mesghali et al. 1967, Javadian and Mesghali 1974, Javadian et al 1976, Seyedi-Rashti and Salehzadeh 1990). In the present study, 15.6% of the vector species were infected. Comparison of the present findings with those obtained from Iran and other countries (Bray 1974, Janini et al. 1995) showed exceptionally high natural infection rates with promastigotes in *P. papatasi* from rodent burrows in Badrood of central Iran.

We confirmed that the highest infection rate of *P. papatasi* occurred in mid-September coinciding with the large second peak of the sand fly abundance (Yaghoobi-Ershadi and Akhavan 1999). As the greatest incidence of human infection occurs in November and December in the area, the time lag between these peaks suggests that the average incubation period of the disease is one to three months.

Phlebotomus papatasi appeared to be an opportunistic feeder in the Badrood area with a wide range of natural hosts including humans, cows, sheep, goats, rodents, and birds. Previous studies on the host preference of *P. papatasi* in Borkhar, Isfahan province suggested that its anthropophilic index varied between 12.7% and 44.4% (Javadian et al. 1977, Yaghoobi-Ershadi et al. 1995b). One possible explanation for the relatively small percentage of human feedings (31.2%) is that many people residing in the study area use bednets or sleep inside air conditioned rooms during the summer months. The Badrood area, which until recently

was unknown as an endemic area seems now to represent a very active focus of ZCL transmission in Iran.

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