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MONTHLY VARIATION OF *LEISHMANIA MAJOR* MON-26 INFECTION RATES IN *PHLEBOTOMUS PAPATASI* (DIPTRA : *PSYCHODIDAE*) FROM RODENT BURROWS IN BADROOD AREA OF IRAN

M.R. YAGHOOBI-ERSHADI, Ph.D., A.A. AKHAVAN,* M.S.P.H., AND M. MOHEBALI, Ph.D.**

*From the Department of Medical Entomology and Vector Control, the **Department of Medical Parasitology, School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences, P.O.Box 6446-14155, Tehran, and *Isfahan Health Training and Research Center, School of Public Health and Institute of Public Health Research, Isfahan University of Medical Sciences, P.O.Box 334-81465, Isfahan, Iran.*

ABSTRACT

Following an epidemiological survey of zoonotic cutaneous leishmaniosis (ZCL) in some 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. Sandflies were collected and dissected biweekly from rodent burrows during sandfly season, April - October 1998. Leptomonad infection rates varied between 6.7-22.0% during sandfly season, being greatest in September, coinciding with peak activity of *P. papatasi*, 2-3 months before the highest incidence of ZCL human cases in November-December. Leptomonad infection rate was 1.1% in 94 *P. papatasi* indoors.

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INTRODUCTION

Zoonotic cutaneous leishmaniosis (ZCL) is endemic in many rural areas of Iran, in 11 of the 28 provinces. In recent years a new focus of ZCL was found in villages of Badrood rural district, Natanz county among the foothills of Karkas mountains in central Iran. A study of prevalence among 3119 inhabitants showed a rate of 27% for active lesions and 2.3% for scars in three villages in 1995.[1]

The main vector for humans is *Phlebotomus papatasi* Scopoli (Diptera: *Psychodidae*) and other vectors among rodents in the country have been reported as *Phlebotomus alexandri* Sinton, *P. (Pa.) andrejevi* Shakirzyanova, *P. (Pa.) caucasicus* Marzinowsky, *P. (Pa.) mongolensis* Sinton and *P. (Synphlebotomus) ansarii* Lewis.[2-6] The following six species of sandflies were found in Badrood rural district: *P. papatasi, P. caucasicus, P. mongolensis, P. alexandri, P. sergenti,* and *S. sintoni. Phlebotomus papatasi* is the most predominant sandfly. This species constitutes 94.1% and 61.4% of the total sandflies captured indoors and in rodent burrows respectively in infected villages in this area.[7] *Rhombomys opimus* and *Meriones libycus* are the main reservoir hosts of ZCL in the district.[8]

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,[4-5] but nothing further 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* and its monthly variation in the ZCL focus of Badrood rural

Correspondence: M.R. Yaghoobi-Ershadi, Department of Medical Entomology, School of Public Health, Tehran University of Medical Sciences, P.O.Box 6446-14155, Tehran, Iran, Telephone: (0098 21) 611-2878, Fax: (0098 21) 646-2267, E-mail :rezatr12@yahoo.com.

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district for the first time in Iran.

MATERIAL AND METHODS

Study area

The investigation was carried out from April to October 1998, for a period of 7 months 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 1056 m., among the foothills of the Karkas mountains (altitude 3898 m.).The area has a desert climate, very hot in the summer and quite cold in the winter. In 1998, the maximum and minimum mean monthly temperature was 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 sandflies were collected biweekly from outdoor resting places (gerbil and jird burrows) and indoors (bedrooms, stables, ...) with the aid of 30 sticky traps. The traps were installed before sunset and collected before sunrise on the next day. The flies were also collected by aspirators from inside resting places. All fed gravid and semigravid females were dissected in a fresh drop of sterile normal saline (9g NaCl /1000mL) and examined microscopically for natural 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 tails of 2 Balb/c mice. Parasites were later reisolated from infected mice and cultured in 2-4 tubes of Novy-Nicolle-MacNeal (NNN) plus Liver Infusion Tryptose (L.I.T.) biphasic medium with penicillin (5×10^6 per mL).

Culture tubes were incubated at 20°C and subcultured every 15 d. All female sandflies were mounted in puri's medium[9] and identified after 48 hrs by the morphology of the pharyngeal armature and spermatheca.[10] The physiological age of each female was determined by the presence or absence of granules in the accessory glands.[11]

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

RESULTS

From May until late October 1998, biweekly sticky trap collections in three villages (60 traps/ village /month) yielded totals of 141 *P. papatasi*, 2 *P.caucasicus* and 17 *S. sintoni* from the vicinity of rodent burrows. Dissection results showed that 15.6% of *P. papatasi* and 5.9% of *S. sintoni* were infected with leptomonads. No leptomonad was found in *P. caucasicus*.

Phlebotomus papatasi infections began to appear in mid May, peaked in the middle of September with a rate of 22.0% and fell to zero at the end of October. Besides gut infection, leptomonads were seen in the esophagus of 59.1% of the positive flies and in the head of 22.7 % of the positive *P. papatasi* (Table I).

The results of examining the accessory glands of the female flies showed that the highest parous rate (96.6% *P. papatasi*) occurred in old populations towards the end of a given generation (September). The highest rate of parasite infestation in *P. papatasi* (22.0%) was observed when the parous rate was high.

Table I. Monthly variation of leptomonad infection rate in *P. papatasi* from rodent burrows, Badrood rural district, Isfahan province, Iran, 1998.

Month	No. Dissected	Age group			No. of sandflies with leptomonads				
		N	P	?	T	G	E	H	%Infected
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 sandflies from nulliparous ones was impossible.

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Promastigotes from 13 heavily infected *P. papatasi* (collected from rodent burrows) were injected subcutaneously into the tail-bases of 2 Balb/c mice each. Six of these 26 mice (23.1%) became infected and nodules and ulcers containing numerous amastigotes developed at the site of inoculation 60-150 d after injection.

Promastigotes grew well by 5d after inoculation in 8 of the 20 N.N.N. + L.I.T. cultures inoculated with the parasites from infected mice. The other 12 cultures were contaminated but no promastigote was seen. The isolate was characterized as *L. major* zymodeme MON-26.

Out of 94 *P. papatasi* collected from indoor resting places in September 1999, only one specimen (1.1%) was found to be infected.

DISCUSSION

This is the first report of 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 Imamzadeh Agha-Ali-Abbas in Iran. Thousands of people visit this sacred place during the active season of sandflies and all of them are exposed to the disease. *Leishmania major* zymodeme MON-26 is known as the agent of ZCL from Sub-Saharan Sahel to the Near and Middle East.[12] In 1995 we isolated the same zymodeme from *M. libycus* and humans in this area[1,8] and also from *P. caucasicus* and *P. papatasi* in Borkhar, a rural district north of the city of Isfahan.[4,5]

Leishmanial infection rates of *P. papatasi* from rodent burrows of other ZCL foci in Iran (i.e. Abardezh, Ahwaz, Dezful, Isferayen, Lotfabad, Shush, Turkemen-Sahra and Isfahan) ranged from 0.2% to 10.9% during 1967-1991[5,13-16] whereas in the present study 15.6% of this species was infected. Comparison of the present finding with those obtained from Iran and other countries,[17,18] 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 sandfly abundance.[7] 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 1-3 months.

This is definite evidence incriminating *P. papatasi* as the vector of *L. major* in this part of Iran. By the typing of isolates from flies, *P. papatasi* also transmits the parasite in ancient U.S.S.R., eastern Saudi-Arabia, southern Morocco and central Tunisia.[19]

Up to now the Badrood area was unknown as an endemic zone; this region seems to represent a very active focus of ZCL transmission in the country.

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