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

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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.


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. 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) ansarri Lewis. 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. Rhombomyos opimus and Meriones libycus are the main reservoir hosts of ZCL in the district.

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, 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...

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Leishmania major monthly variation 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. 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. Para­infusion Tryptose (L.I.T) biphasic medium with penicillin and streptomycin were given generation (September). The highest rate of parasite infection 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, leptomonalons 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 1).

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 1. Monthly variation of leptomodal infection rate in P. papatasi from rodent burrows, Badrood rural district, Isfahan province, Iran, 1998.

<table>
<thead>
<tr>
<th>Month</th>
<th>No. Dissected</th>
<th>No. of sandflies with leptomonalons</th>
<th>Age group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>P</td>
<td>?</td>
</tr>
<tr>
<td>May</td>
<td>14</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>June</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>July</td>
<td>15</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>August</td>
<td>29</td>
<td>5</td>
<td>23</td>
</tr>
<tr>
<td>September</td>
<td>59</td>
<td>0</td>
<td>57</td>
</tr>
<tr>
<td>October</td>
<td>23</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>Total</td>
<td>141</td>
<td>10</td>
<td>124</td>
</tr>
</tbody>
</table>

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.
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 3d 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. In 1995 we isolated the same zymodeme from *M. libycus* and humans in this area and also from *P. caucasicus* and *P. papatasi* in Borkhar, a rural district north of the city of Isfahan.

Leishmanial infection rates of *P. papatasi* from rodent burrows of other ZCL foci in Iran (i.e. Abardez, Ahwaz, Dezful, Isfaryan, Lotfabad, Shush, Turkemen-Sahra and Isfahan) ranged from 0.2% to 10.9% during 1967-1991 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 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. 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.

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.

**ACKNOWLEDGEMENT**

We thank the leishmaniosis staff of the Isfahan Health Training and Research Center for assistance in collecting of sandflies. We are also grateful to Prof. J.P. Dedet and Dr. F. Pratlong (School of Medicine, University of Montpellier) for the typing and characterization of the *L. major* isolate. This investigation received financial support from the Institute of Public Health Research (Tehran University of Medical Sciences), via Project ID No 241.70.20.

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