Epidemiological study of reservoir hosts in an endemic area of zoonotic cutaneous leishmaniasis in Iran

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The study was carried out in the central part of the Islamic Republic of Iran over a 12-month period in 1991–92 in Borkhar, a rural district lying north of Isfahan city. The objective was to determine the ecology of natural reservoir hosts of leishmaniasis for possible future field trials of leishmania vaccine. The main reservoir host in this area is Rhombomys opimus, *the great gerbil, followed by* Meriones libycus, *the Libyan jird, and* Hemiechinus auritis, *the long-eared hedgehog.*

Of the 179 small mammals examined in the Borkhar area, the great majority were R. opimus *(82.1%), then* M. libycus *(15.7%), and last* H. auritis *(2.2%). The highest rate of infection of* R. opimus *was in September (90.5%), the rate varying between 22.2% and 80.4% in different villages. The average infection rate of* M. libycus *was 17.9%. These rodents probably play an important role as reservoir hosts in the epidemiology of zoonotic cutaneous leishmaniasis in this area. Sixteen domestic and stray dogs appeared to be uninfected because examination showed no active lesion or scar.*

Introduction

Zoonotic cutaneous leishmaniasis (ZCL) is endemic and very common in many rural areas of the Islamic Republic of Iran. *Rhombomys opimus*, the great gerbil, is the main animal reservoir in foci in the north-east and central part of the country; in the south-west, beyond the geographical distribution of *R. opimus* in the province of Khuzistan, the main animal reservoir is *Tatera indica*. In the southern part of Iranian Baluchistan, bordering Pakistan, the animal reservoir has been identified as *Meriones hurrianae* (*1–8*).

Phlebotomus papatasi, the most important species of the genus *Phlebotomus*, is the only known vector to humans and gerbils in these foci. The parasite has been identified as *Leishmania major* (*6*, *9*, *10*). Zoonotic cutaneous leishmaniasis is a serious and increasing public health problem in the rural areas that lie north and east of Isfahan city in central Islamic Republic of Iran. Enzyme characterization of numerous isolates from *R. opimus* and patients with cutaneous leishmaniasis has revealed that *L. major* zymodeme LON-1 is widespread in this area (*6*, *10*).

In 1991, we isolated *Leishmania* (Kinestoplastida: family Trypanosomatidae) promastigotes from *P. papatasi* and *P. caucasicus* collected from rodent burrows in the villages of Borkhar, a rural district lying north-east of Isfahan city. They were typed at the Faculty of Medicine in Montpellier, France, by the examination of 15 isoenzymes and identified as *L. major* Yakimoff & Schockov zymodeme MON-26 (*10*, *11*).

Human prevalence of the disease and the incidence of new cases remain high despite passive case detection and treatment by well-trained staff in local clinics and public health services. House spraying with DDT, poisoning of gerbils, and application of DDT powder in rodent burrows have failed to control ZCL in this area (*12–14*).

The study was carried out in four villages in Borkhar district in 1991–92 to determine the ecology of natural reservoirs and their relative importance and to obtain up-to-date information on the local epidemiology prior to future field evaluations of killed *Leishmania major* (KLm) vaccine[a] in this hyperendemic focus.

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Materials and methods

Characteristics of the study area

The investigation was carried out over a period of 12 months (from 21 March 1991 to 20 March 1992) in four villages (Komshecheh, Parvaneh, Aliabadchi and parts of Habibabad; total population, 6284) in the district of Borkhar, 30–48 km north of Isfahan city. The selected villages are part of an endemic focus of leishmaniasis in Isfahan. The soil of the area is generally a little salty, and in some parts sandy (*1*). However, wheat, barley, cotton, vines, beetroot, pistachio, alfalfa, Indian corn, clover and summer crops are cultivated. The underground water table is about 80 m deep in this area (data from General Office of Agriculture, Isfahan Province), which has a desert climate, and is very hot in summer and cold in winter. In 1991, the maximum and minimum mean monthly temperatures were 27.9 °C and −1.2 °C in June and December, respectively. The total annual rainfall in 1991 was 104.8 mm — minimum of 3 mm in March and maximum of 27.4 mm in December. The minimum mean monthly relative humidity was 24.5% (August) and the maximum was 68% (December and January).

Methods

The selected villages were located about 1–1.5 km from the colonies of gerbils. These rodents were trapped from the colonies using 40 live traps once a month by two experienced field technicians, and transferred to the leishmaniasis laboratory of Isfahan Training and Research Station. The gerbils were held from the back of the neck with forceps and from the tail by hand. Their ears, regardless of any obvious lesions, were cleaned with alcohol and scraped using a clean piece (2 cm × 2 cm) of coarse sandpaper. The surface of a clean slide was then pressed onto the scraped part of the ear and moved along to produce a smear on the slide. The slides were fixed with methanol, stained with standard Giemsa, and examined carefully under high magnification for an hour or more to look for amastigotes (*15*). Six smears were prepared and examined from each rodent.

Samples from infected rodents were injected at the base of the tail of outbred or inbred (Balb/c) mice to identify the parasite. Household and stray dogs were physically examined for the presence of any active lesion or scar in a house-to-house survey.

Results

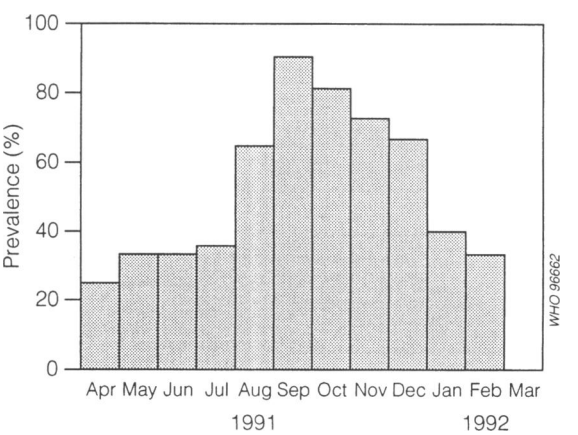
A total of 179 small mammals were captured and examined. Predominant among the three species of small mammals found in the Borkhar area is *R. opimus*, the great gerbil (82.1%); the other two are *Meriones libycus*, the Libyan jird or gerbil (15.7%), and *Hemiechinus auritis*, the long-eared hedgehog (2.2%). *R. opimus* and *M. libycus* are rodents in the subfamily Gerbillinae and *H. auritis* is an insectivore of the subfamily Erinaceinae, family Erinaceidae.

There are many active colonies of *R. opimus* in the area, especially around the eastern part of Habibabad city. The characteristic vegetation is *Salsola* and many small trees (*Haloxylon*) have been planted by the Organization of Forests and Pastures to prevent movement of the flowing sands.

Both *R. opimus* and *M. libycus* were found to be infected with *Leishmania*; 80 out of 147 *R. opimus* (54.4%) and 5 out of 28 *M. libycus* (17.9%) were positive. Leishmanial infection of *R. opimus* was found in all months during the study except for March. The highest rate of infection was 90.5% in September and the lowest was 25% in April (Fig. 1), which was statistically significant ($P < 0.01$). Of the 80 infected animals, 41 showed leishmanial infection on one ear and 39 on both ears. Most of the *R. opimus* examined were infected on both ears during September to November. The infection rate varied between 22.2% and 80.4% for *R. opimus* and 16.7% and 20% for *M. libycus* in different villages. The parasites were almost always scanty. Parasites from infected great gerbils were injected subcutaneously at the base of the tail of 32 laboratory mice (souris) and 9 Balb/c mice. Nodules and ulcers appeared after 18 days in souris and 7 days in the Balb/c mice.

Sixteen domestic and stray dogs were examined and none of them appeared to be infected.

Fig. 1. **Percentage prevalence of leishmanial infection among *Rhombomys opimus* gerbils in Borkhar district, Islamic Republic of Iran, by month, during 1991–92.**



Discussion

Based on this survey, the great gerbil (*R. opimus*) appears to be the main reservoir host of zoonotic cutaneous leishmaniasis in this part of the Islamic Republic of Iran (area, 150 km × 35 km). The results showed that over 54% of these animals had *L. major* infection, which agrees with studies carried out in 1968 (*1*). Close contact between infected *R. opimus* and the human population makes it easy for infected sandflies to feed on humans and transmit the infection.

The main human–gerbil vector is *P. papatasi*; *P. caucasicus*, *P. mongolensis* and *P. ansarii* act as vectors between rodents. This finding confirms previous data obtained by our group (*10*, *11*, *16*) and others (*1*). The highest infection rate in sandflies appeared in late August and September, accompanying the second and largest peak of sandfly activity in the area (*16*). This increased rate of infectivity among sandflies during these months seems to be due to close contact with many infected hosts.

Young *R. opimus* gerbils become adults after the winter, and 25–33.3% of them are infected at the beginning of the active season of sandflies. Breeding starts in early spring and the population grows for 3 months. Transmission in this area begins when the sandflies emerge in late May, but cannot keep pace with the rising rodent population so that the proportion of infected sandflies declines. From July to September the increase in the numbers of *Rhombomys* declines and the proportion of infected animals rises; the highest rate of *R. opimus* infection is therefore in September (Fig. 1) and the chances of infection in sandflies that bite them is then greater. About 62% of the inhabitants showed a scar of leishmanial infection. The high risk of contracting the disease from infected sandflies is due to the existence of numerous rodent burrows.

In our study of *M. libycus*, 5 rodents out of 28 (17.9%) were infected, which is a much higher rate than that found in an earlier study in 1968 (only 1 infected out of 38 (2.6%)) (*1*). Our result may be related to the technique we used to prepare smears from small mammals for the detection of *Leishmania* parasites, and also due to an increase in the rate of infectivity. The present level of leishmanial infection in this rodent shows that this animal also may play an important role as a reservoir host in the epidemiology of zoonotic cutaneous leishmaniasis in this area.

It seems that hyperendemic zoonotic cutaneous leishmaniasis will remain a major public health problem until new methods of intervention, such as an effective vaccine (*17*),[b] can be implemented.

[b] See footnote *a* on page 587.

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Résumé

Etude épidémiologique des hôtes réservoirs dans une zone d'endémie de la leishmaniose cutanée zoonosique en Iran

L'étude a été réalisée sur une période de douze mois en 1991–1992 à Borkhar, un district rural situé au nord d'Ispahan, dans le centre de l'Iran. Elle avait pour objectif de déterminer l'écologie des hôtes réservoirs naturels de la leishmaniose en vue d'éventuels essais sur le terrain d'un vaccin antileishmaniose. Les principaux hôtes réservoirs rencontrés dans la région sont *Rhombomys opimus*, une gerbille, *Meriones libycus*, un mérion, et *Hemiechinus auritis*, un hérisson.

Sur 179 petits mammifères examinés dans la région de Borkhar, la plupart étaient des *R. opimus* (82,1%), suivis de loin par *M. libycus* (15,7%) et *H. auritis* (2,2%). Le plus fort taux d'infection de *R. opimus* a été observé en septembre (90,5%) et variait de 22,2% à 80,4% d'un village à l'autre. Le taux moyen d'infection chez *M. libycus* était de 17,9%. Ces rongeurs jouent probablement un rôle important en tant qu'hôtes réservoirs dans l'épidémiologie de la leishmaniose cutanée zoonosique dans la région. Seize chiens domestiques et errants examinés n'ont en revanche montré aucun signe d'infection (ni lésion ni cicatrice).

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