Annals of Tropical Medicine & Parasitology, Vol. 97, No. 6, 593–603 (2003)

Leishmania major infection in the fat sand rat *Psammomys obesus* in Tunisia: interaction of host and parasite populations

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Received 30 April 2003, Accepted 10 June 2003

The causative agent of cutaneous leishmaniasis, *Leishmania major*, was studied in a Tunisian population of the fat sand rat, *Psammomys obesus*. Seasonal changes in the abundances of parasite and host were monitored in a longitudinal field survey lasting 21 months. Overall, 566 *P. obesus*, collected during 10 trapping sessions between May 1995 and January 1997, were examined. Analysis of prevalence, using logistic regression, revealed that extrinsic factors, such as season and climatic conditions, and intrinsic factors, such as host age, have a combined effect. *Leishmania major* showed a seasonal pattern, with most transmission occurring in late summer and in autumn, when prevalences were 7.5- and 6.6-fold higher, respectively, than in spring. Prevalence peaked, at 70%, in September 1995 and then decreased to 0% in June 1996. The same temporal fluctuation was observed during the second study year, albeit among prevalences that were relatively low because of unusually dry conditions. Prevalence was highly dependent on the age of the *P. obesus*, and season and age acted in synergy so that the rodents were highly infected in late summer and in autumn. Prevalence was not correlated with the relative density of the *P. obesus* and also appeared independent of gender. Although the ear lesions observed on 378 sand rats during a 1-year survey were closely associated with *Leishmania* infection, such lesions were not good predictors of infection, as 35% of the rodents found to be infected had no visible lesions on their ears. The prevalences of *Leishmania* infection observed in this study, among *P. obesus* living in monospecific colonies, were generally lower than those observed in other studies of *P. obesus*. It seems possible that *P. obesus* living in monospecific colonies could have a lesser role in propagating the parasite than those living in plurispecific colonies of rodents, and act as an 'epidemiological sink'.

Outbreaks of human cutaneous leishmaniasis (HCL) caused by *Leishmania major*, of considerable economic and social importance, have been increasingly reported from North Africa and the Arabian Peninsula since the 1970s. Recent settlers and indigenous people have both been affected.

The fat sand rat, *Psammomys obesus*, is generally considered to be the main reservoir host of *L. major* throughout the North African and south–west Asian distribution of the infection (Ashford, 2000). Gunders *et al.* (1968) first reported *L. major* in *P. obesus*, in Israel. This parasite–host combination was subsequently observed in Libya, with formal identification of the parasites (Ashford *et al.*, 1977), then in Algeria (Belazzoug, 1983), central Saudi Arabia (R. W. Ashford, unpubl. obs.), eastern Saudi Arabia (Peters *et al.*, 1986), Tunisia (Ben Ismail *et al.*, 1987) and Jordan (Saliba *et al.*, 1994).

Psammomys obesus is a medium-sized rodent (with adults weighing 150–200 g) that lives in saline habitats, notably 'succulent halophytic steppes' or along wadi edges, where its main food, plants of the family Chenopodiaceae, grows (Petter, 1961; Kam and Degen, 1989;

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DOI: 10.1179/000349803225001517

Zaime and Gautier, 1989; Fichet-Calvet *et al.*, 2000*b*; Tchabovsky and Krasnov, 2002). It is largely diurnal, lives in densely packed burrows, and reproduces in winter and spring (Amirat *et al.*, 1977; Fichet-Calvet *et al.*, 1999).

The main aim of the present, 21-month study was to describe the interaction between the populations of the *L. major* parasites and their natural *P. obesus* hosts in Tunisia, in the hope that the results would help to explain (and possibly to predict) outbreaks of HCL.

The temporal changes in a Tunisian *P. obesus* population and in the prevalence of *L. major*, in relation to abiotic (season, climatic conditions) and biotic (host gender, age and relative density) factors, were investigated. Incidentally, the reliability of the detection of ear lesions in the diagnosis of *L. major* infection in *P. obesus* was compared with that of mouse-footpad inoculation.

ANIMALS AND METHODS

Study Site and Sampling of Host Population

The study was carried out 40 km south of Sidi Bouzid (35°46′N, 9°36′E; 280 m above sea level), near the village of R'milia. The area is in the arid bioclimatic zone, with mean annual rainfall (1960–1995) of 260 mm, and the study site was on the edge of a *sebcha* — an area of 'succulent halophytic steppe' (Le Houerou and Le Floc'h, 1995). The vegetation at the site, predominantly plants of the family Chenopodiaceae (*Salsola*, *Suaeda* and *Arthrocnemum* spp., with occasional *Atriplex* sp.), represented the much disturbed remnants of the edge of the *sebcha* (Ozenda, 1991). The 100-ha site was traversed by earth bunds some 2 m high, constructed for flood control, between which were cultivated plots of wheat, separated by bands of natural vegetation (Fichet-Calvet *et al.*, 2000*b*). Samples of the local *P. obesus* were collected on 10 occasions between May 1995 and January 1997, at 7- to 13-week intervals (Fichet-Calvet *et al.*, 1999). According to the criteria of Gaussen (Ozenda, 1986), the climatic conditions were wet in the September and November of 1995 and in March 1996 and dry at the other times rodents were trapped (Fichet-Calvet *et* al., 1999).

Host-population Parameters

TRAPPING

Psammomys obesus rarely enters live-traps with conventional bait, but can be captured in cage traps baited with fresh food-plants, placed close to burrow entrances. Two kinds of trap were used in the present study: unbaited pincer traps placed in the mouths of the burrows on the bunds and wire-mesh cage traps placed close to burrow mouths on the bands of residual *sebcha*. Trap lines varied between 60 and 280 m in length, and the traps were left in place for 3 days (Fichet-Calvet *et al.*, 1999). For each round of trapping, the mean number of *P. obesus* captured/50-m line of pincer traps was added to the mean number/50-m line of cage traps (with a trap set in every burrow on each line) to give a relative 'abundance index'.

AGE DETERMINATION

All the *P. obesus* caught were killed, under anaesthetic, by complete cardiac exsanguination. As the weight of the desiccated eye lens is considered to be the best indirect measure of age for mammals, clearly distinguishing young animals from older individuals of similar weight or body size (Lord, 1959; Martinet, 1966; Morris, 1971), eye-lens weight (ELW) was used to age the *P. obesus*. The eyes were removed from each rodent caught, and preserved for at least 2 weeks in 10% formalin before each lens was extracted, dried for 2 h at 100°C and weighed, on a pan balance, to an accuracy of 0.1 mg. ELW was recorded as the combined weight of both desiccated lenses.

CHECKING FOR EAR LESIONS

The ears of each rodent caught between November 1995 and November 1996 were checked for lesions, scars and thickenings.

Detection of *Leishmania* Infection

Both ears of each rodent caught were removed and macerated together in physiological saline. A subsample of the suspension produced was inoculated subcutaneously into a hind footpad of a BALB/c mouse, one mouse being inoculated with the macerated ear tissue of each *P. obesus* (Ben Ismail *et al.*, 1989). The hind footpads of the inoculated mice were examined regularly until a lesion was observed (indicating probable infection with *L. major*) or, if no lesion developed, until 8 months post-inoculation. A mouse that developed a footpad lesion was only declared positive for *Leishmania* after amastigotes had been detected microscopically in smears of dermal scrapings or promastigotes had been observed in NNN cultures set up using a skin sample from the swollen footpad. Although the parasites observed were not formally identified, they were all assumed to be *L. major*.

Data Analysis

For the purposes of the present study, the naming of the seasons was based on the cycle of reproduction in the local *P. obesus* (Fichet-Calvet *et al.*, 1999). For example, the period when the *P. obesus* population was composed of many young animals and a few sexually active adults (May in 1995 and March in 1996) was considered spring. Summer (July 1995 and June 1996) was when all the animals were inactive adults. Reproductive activity recommenced in late summer (September 1995 and August 1996), the first young of the year were born in autumn (November 1995 and November 1996), and young and old adults reproduced in winter (January 1996 and January 1997).

The effects of season, climatic period, and gender, age and relative abundance of the *P. obesus* on the prevalence of *L. major* infection in the rodents were analysed by multiple logistic regression. For this, a binary factor (infected = 1; non infected = 0) was used as the dependent variable and season (spring, summer, late summer, autumn or winter), climatic period (wet or dry), host gender (male or female), host age (as indicated by ELW) and host relative density (measured as the abundance index) were used as the independent variables. The strategy for the data analysis was to enter all the variables in a global model, then to remove the non-significant variables, giving a restricted model, and finally to add the interactions in the restricted model (Kleinbaum and Klein, 2002). Odds ratios (OR) were calculated from the restricted model without the interactions, following the guidelines given by Falissard (2001). This regression analysis was performed using the Systat 9 software package (SAS Institute, Chicago, IL).

The association between visible ear lesions and *L. major* infection (as detected by footpad inoculation) was analysed by multiple logistic regression in which ear lesion was the dependent variable (lesion = 1; no lesion = 0), and confirmed infection status (infected or uninfected), season (spring, summer, late summer, autumn or winter) and age (as indicated by ELW) were the independent variables. This analysis was performed using the Statview 5 software package (SAS Institute). As the goal of this analysis was to obtain a single estimate of the *Leishmania* infection, adjusted for season and age, no interactions were considered (Kleinbaum and Klein, 2002).

RESULTS

A total of 600 *P. obesus* were caught between May 1995 and January 1997; 566 were examined for *L. major* infection and 74 were found positive (Table 1). The only other rodents collected were seven *Meriones shawi* and one *Gerbillus pyramidium*.

Prevalence

The percentage of *P. obesus* caught that were found positive for *L. major* varied with the trapping round (Fig. 1), the prevalence of infection being particularly high in September

TABLE 1. *Distribution of the* Psammomys obesus *captured and examined for* Leishmania major *infection, by month of collection*

Collection month	No. and (%) of *P. obesus*: Captured	Examined	Found infected
May 1995	74	74	0 (0)
July 1995	20	20	1 (5)
September 1995	56	30	21 (70)
November 1995	61	61	13 (21)
January 1996	65	65	8 (12)
March 1996	103	103	5 (5)
June 1996	65	65	0 (0)
August 1996	45	41	7 (17)
November 1996	43	43	19 (44)
January 1997	68	64	5 (8)
All	600	566	74 (13)

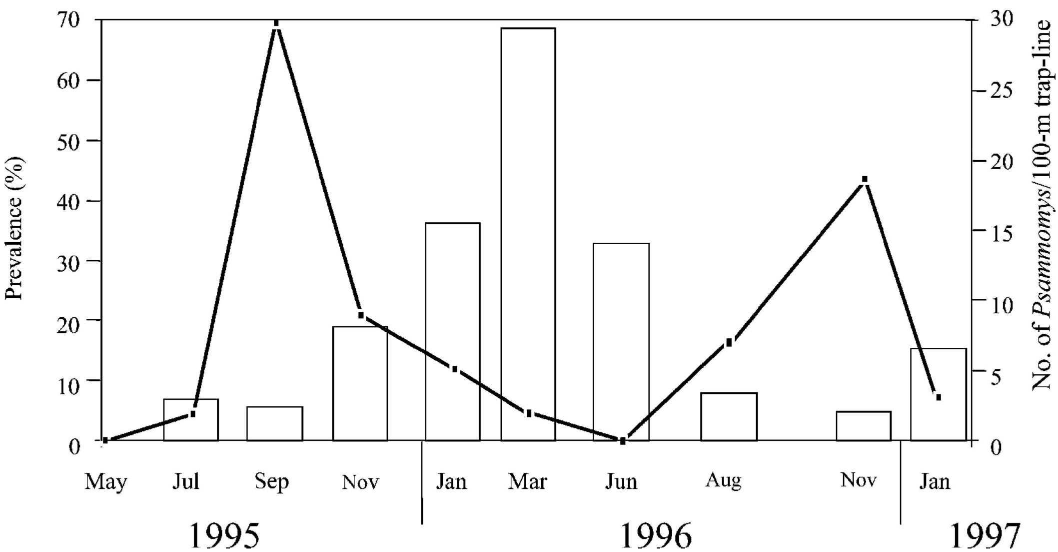

FIG. 1. Seasonal variation in the prevalence of *Leishmania major* in the *Psammomys obesus* examined (line) and the relative abundance of the *Psammomys obesus* (bars).

1995 (70%) and November 1996 (44%), when the host population was at low density. Prevalence decreased as the relative abundance of the *P. obesus* increased, falling to zero in late spring (May 1995 and June 1996). When prevalence was analysed by multiple logistic regression, with season, climatic period, and host gender, age and relative density as sources of variation, the three significant factors appeared to be season ($P < 0.0001$), climatic period (irrespective of season; $P = 0.0017$) and host age ($P < 0.0001$); the gender and relative density of the *P. obesus* were not significant. The *P*-values, partial coefficients and OR estimated when the three significant factors were included in a restricted model are shown in Table 2. The seasons late summer and autumn were found to be positively associated with infection, with corresponding prevalences that were, respectively, 7.5- and 6.6-fold higher than that in spring (considered as the reference in this analysis). Climatic period also affected prevalence, the relevant OR indicating that

TABLE 2. *The logistic-regression results for* Leishmania major *infection among 565* Psammomys obesus *in Tunisia*

Variable	P	Partial coefficient	Odds ratio
Season summer	0.5627	0.000	0.508
Season late summer	0.0006	0.146	7.564
Season autumn	0.0011	0.138	6.662
Season winter	0.4778	0.000	1.615
Climatic period	<0.0001	0.186	4.723
Host age (4.6 mg < eye-lens weight < 62.4 mg)	<0.0001	0.298	1.108

TABLE 3. *The logistic-regression results for ear lesions on 378* Psammomys obesus *in Tunisia*

Variable	χ^2	Degrees of freedom	P
Leishmania major infection	14.026	1	0.0002
Season	25.620	4	<0.0001
Host age (4.6 mg < eye-lens weight < 62.4%mg)	59.094	1	<0.0001

prevalence was 4.5-fold higher in wet conditions than in dry (Table 2). Host age and prevalence appeared correlated ($r = 0.298$; $P < 0.0001$). The two-way 'season × climatic period', 'season × ELW' and 'climatic period × ELW', and three-way 'season × climatic period × ELW' interactions were also all significant ($P < 0.0001$ for each). These results are illustrated in Figure 2, where each trapping round is presented as pyramid of age (using ELW as a proxy for age); clearly older animals account for most of the infections. Only one infected rodent was caught, however, in May (1995), June (1996) or July (1995), even though many of the *P. obesus* collected in these months were adults. In January 1996 and March 1996, a few very young *P. obesus* (with ELW of <20 mg) were found infected, indicating that some transmission of *L. major* to *P. obesus* occurs in winter.

Ear Lesions

The lesions observed on the ears of the fat sand rats were found to be strongly associated with *L. major* infection when season and host age were included in the model (Table 3). Among the *P. obesus* that were checked for ear lesions, however, 35% (18/52) had no visible lesion on the ears although they were found to be infected with *Leishmania*, and 40% (23/57) of those with ear lesions were negative for *L. major* when tested by footpad inoculation (Table 4).

DISCUSSION

Effects of Season, Host Age and Climatic Conditions

The prevalence of *L. major* in *P. obesus* varied greatly through the year, from an undetectable level in late spring to 70% in late summer. These seasonal changes show the importance of a longitudinal study: even in a proven reservoir host, prevalence can be vanishingly low, and comparisons between localities must be adjusted for seasonal effects.

The overall prevalence of 13% observed here is relatively low compared with the

TABLE 4. *Observation of ear lesions and* Leishmania major *infection (as detected by footpad inoculation of BALB/c mice) in* Psammomys obesus

Ear lesion	*Leishmania major infection*	No. of *P. obesus*
Negative	Negative	303
Negative	Positive	18
Positive	Positive	34
Positive	Negative	23

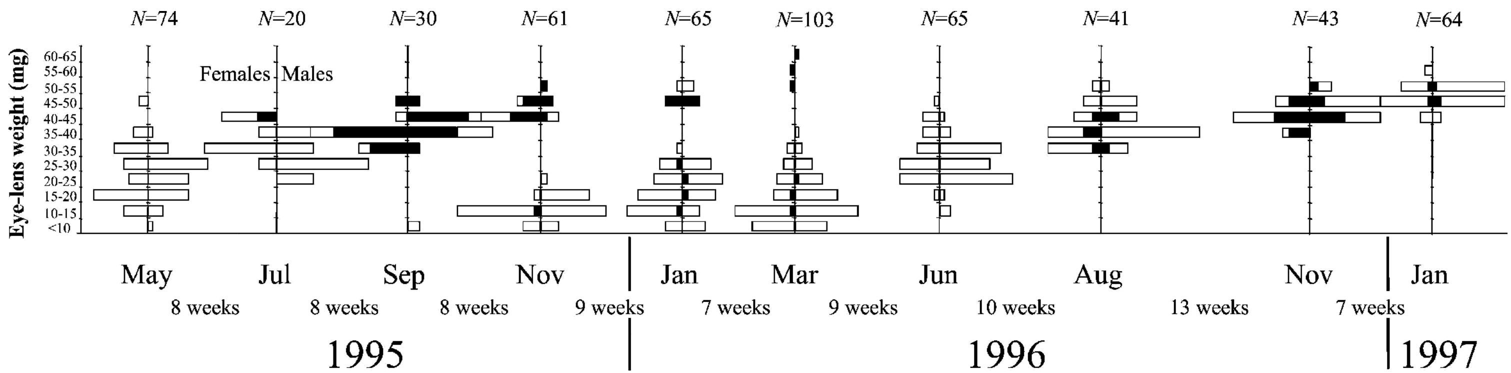

FIG. 2. Distribution of *Leishmania major* in *Psammomys obesus*, split according to the eye-lens weight (as a proxy for age) and gender of the host and by season. Each pyramid corresponds to the proportion of females (left) and males (right) sampled at each trapping round, and shows the percentages found infected (■) and not infected (□). To adjust for the temporal variation in sample size (as indicated, above the pyramids, by the numbers of *P. obesus* caught in each trapping round), the length of each horizontal bar reflects the proportion of individuals in each collection, so that the total length of bars in each collection = 1.

corresponding prevalences observed, following studies that covered several seasons (see Table 5), in Syria (28%; Rioux *et al.*, 1992) and Israel (51%; Wasserberg *et al.*, 2002). Differences in technique may partly explain these differences in prevalence: Rioux *et al.* (1992) only checked *P. obesus* with visible lesions, and Wasserberg *et al.* (2002) used PCR-based methods (which are possibly more sensitive than footpad inoculations) to detect infection. Even using imprints of the ear alone, however, Wasserberg *et al.* (2002) observed an astonishingly high prevalence of 46%, both in late spring (June 1998) and winter (January 1999 and February 2000).

The prevalence of infection among the *P. obesus* caught in spring (March 1996) in the present study, around 5%, was well below those seen, in the same season, by Ashford *et al.* (1977) in Libya or by Rioux *et al.* (1990) in Syria (Table 5). Again, these differences may partly be attributed to the sampling methods, as only animals that showed lesions were checked for infection in the earlier investigations. The spring prevalence of 5% observed in the present study is close to that reported by Belazzoug (1983) in Algeria. Elsewhere in Tunisia, however, in the Douara focus, some 200 km south of Sidi Bouzid, Ben Ismail *et al.* (1987) observed a prevalence in spring of 16% even though they used a less sensitive diagnostic method than footpad inoculation (Ben Ismail *et al.*, 1989). In Israel, Schlein *et al.* (1984) found 7% of the *P. obesus* that they caught in late spring (May) to be infected whereas the apparent prevalence of infection among the *P. obesus* caught at this time of year during the present study was 0%. Clearly, the prevalence of infection in the present study area was lower, at a given season, than was found further south in Tunisia, or in Israel. In all three of the relevant earlier studies (Schlein *et al.*, 1984; Ben Ismail *et al.*, 1987; Wasserberg *et al.*, 2002), however, many rodents other than *P. obesus* were caught and found to be infected (Table 5). These included *M. crassus* (7%–10% infected), *M. libycus* (33%), *M. shawi* (14%) and *G. dasyurus* (16%). In contrast, the present study site was inhabited almost exclusively by *P. obesus* — only seven *M. shawi* and one *G. pyramidum* but 600 *P. obesus* were caught over the 21-month study. It seems possible that where *L. major* has access to a wider range of rodent hosts, the prevalence in each rodent species present is increased. Such enhancement of transmission among plurispecific populations of rodents may occur in areas of Iran where *Rhombomys opimus* and *M. libycus* are both common and frequently infected with *L. major* (Yaghoobi-Ershadi *et al.*, 1996). Although few *Nesokia indica* from south–west Asia have been checked for *Leishmania* infection, this species of rodent can be found infected and may therefore also play a role in the epidemiology of *L. major* in this region (Schlein *et al.*, 1984; Yaghoobi-Ershadi *et al.*, 2001).

In the present study, the highest prevalences of *P. obesus* infection were found in late summer (September 1995 and August 1996), when the relative abundance of the rodents was low and, according to Helal *et al.* (1987), that of sandflies in the study area was high. In Turkmenistan, the prevalence of *L. major* in another rodent host, *Rhombomys opimus*, was found to show a similar seasonal trend, infection fluctuating between 8% in May–June and 55% in August–September (Strelkova *et al.*, 2001). In Tunisia, late summer is probably the period of maximum transmission, with many relatively old sandflies as well as numerous infected *P. obesus*. At this time, following 3 months with no breeding, all the rodents are adults aged at least 4 months (Fichet-Calvet *et al.*, 1999). A positive correlation was observed between *P. obesus* age and prevalence of *Leishmania* infection in the present study and also in the investigations by Ben Ismail *et al.* (1987) in the Douara focus in Tunisia, and by Wasserberg *et al.* (2002) in Israel (although, in the earlier studies, bodyweight, not ELW, was used as an indicator of age).

TABLE 5. *Reservoir hosts for* Leishmania major, *with the date of the study, the method used to detect infection, the organ investigated and prevalence observed in each survey*

Reservoir	Country	Date	Detection method	Organ sampled	Prevalence (%) and (no. infected/ no. examined)	Reference
Gerbillus dasyurus	Israel	June 1998–February 2000	PCR	Ear	16 (12/74)	Wasserberg *et al.* (2002)
Gerbillus pyramidum	Egypt	September 1989–June 1991	Smear, biopsy culture	Skin, spleen	<1 (1/128)	Fryauff *et al.* (1993)
Meriones crassus	Israel	May 1980	Biopsy culture	Ear, spleen, liver	10 (15/155)	Schlein *et al.* (1984)
	Israel	June 1998–February 2000	PCR	Ear	7 (2/28)	Wasserberg *et al.* (2002)
Meriones libycus	Libya	April 1976	Smear	Ear	25 (1/4)	Ashford *et al.* (1977)
	Tunisia	February 1987–April 1987	Smear	Ear	33 (2/6)	Ben Ismail *et al.* (1987)
	Saudi Arabia	January 1992–December 1992	Smear	Ear	14 (30/94)	Ibrahim *et al.* (1994)
	Iran	October 1994–October 1995	Smear	Ear	25 (9/36)	Yaghoobi-Ershadi *et al.* (1996)
	Iran	December 1997–May 1998	Smear	Ear	7 (2/28)	Yaghoobi-Ershadi *et al.* (2001)
Meriones shawi	Morocco	December 1981	Lesion, biopsy culture	Ear	7 (2/28)	Rioux *et al.* (1982)
	Tunisia	April 1984	Biopsy culture	Ear	2 (3/121)	Rioux *et al.* (1986)
	Tunisia	February 1987–April 1987	Smear	Ear	14 (2/14)	Ben Ismail *et al.* (1987)
Nesokia indica	Israel	May 1980	Biopsy culture	Ear, spleen, liver	22 (2/9)	Schlein *et al.* (1984)
	Iran	December 1997–May 1998	Smear	Ear	50 (1/2)	Yaghoobi-Ershadi *et al.* (2001)
Psammomys obesus	Lybia	April 1976	Smear	Ear	50 (4/8)	Ashford *et al.* (1977)
	Algeria	April 1982	Smear	Ear	5 (3/60)	Belazzoug (1983)
	Israel	May 1980	Biopsy culture	Ear, spleen, liver	7 (4/54)	Schlein *et al.* (1984)
	Tunisia	February 1987–April 1987	Smear	Ear	16 (15/96)	Ben Ismail *et al.* (1987)
	Syria	May 1989	Lesion	Ear, nose	62 (38/61)	Rioux *et al.* (1990)
	Syria	May 1990–November 1991	Biopsy culture	Ear, nose	28 (33/117)	Rioux *et al.* (1992)
	Jordan	?	Biopsy culture	Ear	23 (39/170)	Saliba *et al.* (1994)
	Israel	June 1998–February 2000	Smear, PCR	Ear	51 (68/133)	Wasserberg *et al.* (2002)
Rhombomys opimus	Iran	November 1979	Smear	Ear	5 (5/103)	Zovein *et al.* (1984)
	Iran	September 1980	Smear	Ear	41 (34/87)	Zovein *et al.* (1984)
	Iran	October 1994–October 1995	Smear	Ear	32 (8/25)	Yaghoobi-Ershadi *et al.* (1996)
	Uzbekistan	June 1983–September 1988	Biopsy culture	Ear	24 (54/225)	Strelkova *et al.* (2001)
	Turkmenistan	June 1991–August 1995	Biopsy culture	Ear	27 (56/205)	Strelkova *et al.* (2001)

In the present study, the eight young animals found infected in November 1995 and January 1996, and especially the three found infected in March 1996, indicate that the local transmission season is longer than had been suspected. The infected rodents caught in March must have been born in January or February, when no adult sandflies have been found. It seems that a few infected sandflies survive well into the winter, possibly inside the burrows. Transmission in the winter of 1996–1997 could not be confirmed because the *P. obesus* had not begun to breed by the time of the final collection.

The apparent absence of *P. obesus* infection in late spring (in both 1995 and 1996) raises the question of how *L. major* survives from one year to the next. At this time, the animals that would be expected to be infected are those born more than 1 year earlier (plus a few that were infected during the previous autumn and winter), that had lived through a transmission season. The present study covered two transmission seasons, the second of which was much drier than the first (Fichet-Calvet *et al.*, 1999). The wetter conditions in the first year favoured the breeding of the rodents (Fichet-Calvet *et al.* 1999), and led to the construction of many new burrows, possibly favouring the survival and reproduction of the sandflies.

Ear Lesions as Indicators of Infection

Lesions on the ear proved to be a good, but by no means perfect, diagnostic sign. Of the *P. obesus* confirmed to be infected, 35% showed no lesions on their ears. The infected animals that did not have lesions may have been those with recently acquired infections. It is also possible that the inflammatory reaction to *L. major* is simply less intense in *P. obesus* than in some other host rodents, such as *M. shawi*, which generally develops large lesions on its ears and nose (Rioux *et al.*, 1982, 1986). Many (40%) of the *P. obesus* with ear lesions were negative for *L. major*. Ear lesions may not only be caused by *Leishmania* but also by fighting (Gromov, 2001) and by the bites of ticks or fleas. That the *P. obesus* at the study site are frequently infected with *Bartonella*, *Babesia* and *Borrelia* species (Fichet-Calvet *et al.*, 2000*a*) indicates that the rodents are exposed to many blood-feeding arthropods. The unreliability of ear lesions in diagnosis indicates the need for a thorough investigation of all animals, for the quantification of *Leishmania* prevalence.

General Comments

The Sidi Bouzid study site is close to the northern limit of the distribution of both *P. obesus* and human infection with *L. major*. Anecdotal observations indicate that *P. obesus* populations vary enormously, in pluri-annual fluctuations. They benefit from vegetation growth when rainfall is plentiful, but are severely reduced by flooding. It is tempting to suppose that Sidi Bouzid is an epidemiological ‘sink’ area (Lidicker, 1975) for the parasite, which becomes extinct locally from time to time. This would help to explain the explosive nature of the Tunisian epidemic of HCL described by Ben Ismail and Ben Rachid (1989). In the second winter of the present study, *P. obesus* reproduction had not started in the final sampling period, in January 1997; in each of the 2 years investigated, *L. major* infection became very rare in the *P. obesus* in late spring, when the animals that had survived the previous transmission season were nearly all dead, and younger animals had had little chance to become infected. Clearly, winter conditions at this latitude are marginal for the survival of the parasite. Further south, even in Tunisia, it is probable that, with warmer winters, the sandflies have a longer period of activity. This, along with the greater diversity of rodent hosts, should allow more assured survival of the parasite suprapopulation, in ‘source’ conditions. It is in the more marginal habitats that some of the epidemics have occurred. If such habitats are truly ecological sinks, then it should be relatively easy to limit transmission in them.

ACKNOWLEDGEMENTS. This study was supported by the Avicenne Programme of the European Commission, AVI CT92/0018. The *Laboratoire d'Epidemiologie et d'Ecologie Parasitaire* in Tunis was also financed for this project by the UNDP/World Bank/ WHO Special Programme for Research and Training in Tropical Diseases and by the International Development Research Centre in Canada.

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