Comparative infectivity of a Kenyan strain of "Leishmania donovani" amastigotes for "Rattus rattus" and the laboratory white rat

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Summary

Amastigotes of a Kenyan strain of *Leishmania donovani* from a previously infected hamster were used to inoculate *Rattus rattus* and the laboratory white rat intracardially. The animals were sampled at 2, 4, 6, and 12 weeks post-inoculation to determine infectivity and total parasite burdens in the liver and spleen. Higher parasite burdens were observed in the livers and spleens of *R. rattus*. Parasite culture indicated more generalized parasite dissemination compared to the white rat. Demonstration of the parasite in dermal tissue of *R. rattus* at 2 and 4 weeks suggests that the parasite may be accessible to sandfly vectors. Transient susceptibility to systemic infection and parasite survival in dermal tissue suggests a potential role of *R. rattus* in the transmission cycle of Kenyan visceral leishmaniasis.

Key words: *Rattus* spp.; *Leishmania donovani;* parasite burdens; parasite culture; epidemiology.

Introduction

Experimental infections of wild-caught rodents with pathogenic strains of *Leishmania* spp. have been used in evaluating susceptibility of the captive rodent and its potential usefulness as a model for the study of the human disease phenomena caused by *Leishmania* (Mikhail and Mansour, 1973; McKinney and Hendricks, 1980). Experimental infections also provide valuable information on the reservoir potential of the rodents under investigation (Heyneman and Mansour, 1963; Stauber et al., 1966; Al Taqi and Mohammed, 1981; Gra-

doni et al., 1983). During the course of experimental infection, some characteristics of a "good reservoir", including host susceptibility (Bray, 1982), can be evaluated. Although parasite isolation from mammals in the field is an important criterion for implicating and designating reservoir status in animals, this approach is not without its limitations (Heyneman and Mansour, 1963). Behaviour of parasite strains in suitable experimental hosts similarly provides an important tool in the biological characterization of pathogenic leishmanial organisms (Stauber, 1966; Mansour et al., 1970; Khairy and El-Hashimi, 1980; Al Taqi and Mohammed, 1981).

Rattus rattus is a widely distributed rodent species from which *L. donovani* has been isolated (Hoogstraal et al., 1963; Bettini et al., 1980) and which is capable of supporting parasite replication in visceral organs after experimental infection (Heyneman and Mansour, 1963; Gradoni et al., 1983). The laboratory white rat is readily available and has been shown to be resistant to experimental infection with *L. donovani* (Stauber, 1958; Giannini, 1985). This study was undertaken to investigate the comparative infectivity of a Kenyan strain of *L. donovani* to *R. rattus* and the laboratory white rat and to evaluate parasite distribution in the tissues of the two rat species.

Materials and Methods

Parasites. Amastigotes of *Leishmania donovani* (strain NLB 065), originally came from a visceral leishmaniasis patient and were subsequently maintained by intracardiac hamster to hamster passage. Amastigotes for this study were obtained from the spleen of a previously infected hamster in the 13th passage. Amastigotes were isolated by the method of Stauber et al. (1958). The resulting amastigote suspension was adjusted to a final concentration of 2×10^8 parasites/ml for subsequent infection of the experimental rats.

Infection of rats with amastigotes. A suspension of 2×10^7 amastigotes in 0.1 ml normal saline was inoculated intracardially into each of 8 laboratory-bred weanling littermates of *R. rattus* and laboratory white rats.

Examination of rats for infection and parasite culture. Two rats from each species were killed at 2, 4, 6, and 12 weeks post-inoculation. At each interval, liver and splenic impression smears were prepared for the estimation of parasite burdens (Stauber et al., 1985). Briefly, the number of amastigotes were counted per 1000 nucleated spleen or liver cells. The ratio of amastigotes to nucleated cells was multiplied by the organ weight in mg and a correction factor of 2×10^5. Parasite burden values were used as estimates for parasite burdens for each organ. Portions of each of these organs were used to inoculate Schneider's Insect Medium (Hendricks et al., 1978) supplemented with 20% (v/v) foetal bovine serum (FBS), 500 IU penicillin, 500 μg streptomycin and 250 μg 5-fluorocytosine (Kimber et al., 1981). Portions of the skin from both hind foot-pads, popliteal lymph nodes and needle aspirates of the left femoral bone marrow were similarly used to inoculate Schneider's Insect Medium for parasite culture. Culture flasks were incubated at 26°C and examined daily for promastigotes. Cultures were considered negative if no parasites were detected over a fourteen-day period.

Results

Parasite culture revealed a generalized dissemination of infection in *R. rattus* for the 12-week observation period. Foot-pad cultures were, however,

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Table 1. Parasite cultures in *Rattus rattus* and laboratory rats infected with 2×10^7 *L. donovani* amastigotes administered intracardially

Weeks after infection*	*Rattus rattus***					Laboratory rat**				
	Spleen	Liver	Bone marrow	Lymph nodes*	Skin*	Spleen	Liver	Bone marrow	Lymph nodes*	Skin
2	2/2	2/2	2/2	2/4	1/4	2/2	0/2	1/2	4/4	0/4
4	2/2	2/2	2/2	4/4	2/4	1/2	0/2	0/2	0/4	0/4
6	2/2	2/2	2/2	4/4	0/4	1/2	0/2	0/2	0/4	0/4
12	1/2	1/2	1/2	2/4	0/4	1/2	0/2	0/2	0/4	0/4

 * Two hind foot-pads and 2 popliteal lymph nodes were cultured from each rat.
** Number of cultures positive / number tested.

only transiently positive (Table 1). In addition, total visceral parasite burdens in this species exceeded the initial inoculum during the first 6 weeks post-inoculation, thereafter declining to levels lower than the inoculum dose over the following 6 weeks. A transient increase in the splenic parasite burden after an initial decline was also observed in this species (Fig. 1). Parallel increases in liver (23%) and spleen weights (56%) were also observed during the first 8 weeks post-inoculation.

In contrast, parasite dissemination was only transient in the laboratory rat, with the majority of the tissues cultured remaining negative for most of the observation period. Foot-pad, skin and liver cultures remained negative at all sampling intervals (Table 1). Liver parasite burdens rapidly decreased to levels undetectable by the smear method by 6 weeks after inoculation. Amastigotes in the spleen of the laboratory rat were not detectable in smears at any time during the observation period (Fig. 1). Comparable increases in the spleen or liver weights were not observed during the course of infection in the laboratory rat.

There was no sign of ill-health in any of the two infected rat species. Culture of about 0.5 ml of heart blood on two occasions (6 and 12 weeks post-inoculation) did not reveal parasites in either of the two species.

Discussion

Our observations suggest that *R. rattus* is transiently susceptible to experimental infection with a Kenyan strain of *L. donovani* amastigotes administered intracardially. Visceral parasite burdens decline after an initial rise during the first 4 weeks after inoculation; a tendency towards parasite clearance over a 120–240-day period has been reported in *R. rattus* (Gradoni et al., 1983). However, at termination of the experiment (12 weeks), 50% of the tissues sampled (except the skin) were positive by culture. Parasite dissemination to dermal tissue, which coincided with peak splenic parasite burden, may partially explain
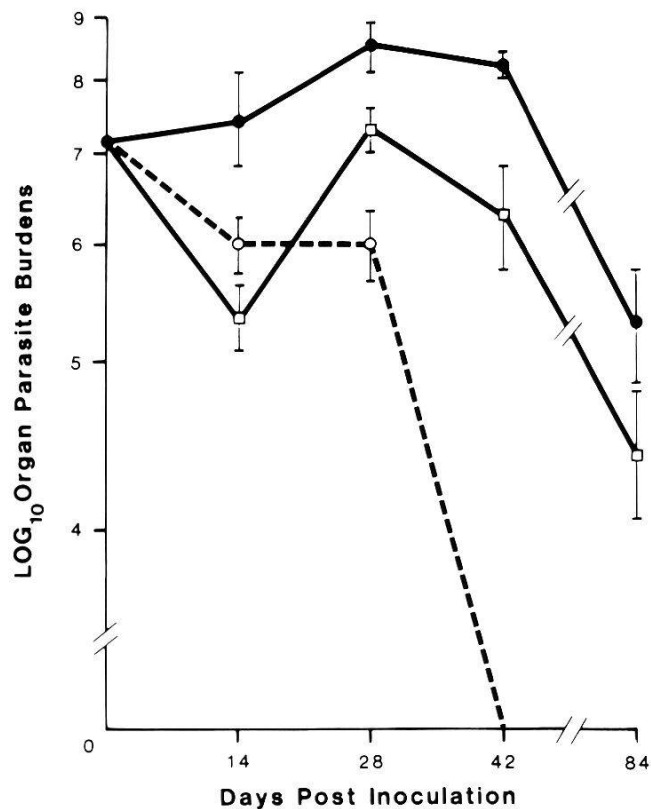
Fig. 1. Parasite burdens in the liver (●—●) and spleen (□—□) of *R. rattus* and liver of the laboratory white rat (○—○) infected intracardially with 2×10^7 amastigotes of *L. donovani.*

enhanced infectivity of rats for sandfly vectors at certain intervals after infection (Pozio et al., 1985).

In contrast, the laboratory rat is more resistant to experimental systemic infection as indicated by the rapid decline of visceral parasite burdens in impression smears and the disappearance of parasites from most tissues cultured during the first 2 weeks of infection. Further, failure to detect promastigotes in foot-pad skin cultures suggests relatively poor parasite survival in dermal tissues. Similar findings have been reported for intradermally inoculated *L. donovani* in *R. norvegicus* (Giannini, 1985). Despite the early disappearance of microscopically detectable visceral infection, the laboratory rat appears capable of sustaining low splenic parasite concentration, detectable by culture, for up to 12 weeks. The detection of a few parasites in liver impression smears but not in culture could possibly be due to poor or non-viability, a phenomenon observed in visceral leishmaniasis patients undergoing chemotherapy (Lightner et al., 1983).

Observations reported here suggest that it may be premature to exclude a potential role of *R. rattus* in the transmission cycle of Kenyan visceral leishmaniasis, in view of their transient susceptibility and ability to localize parasites in foot-pad skin during peak visceral infection, which could render them accessible to vectors. Recent observations confirm susceptibility of *R. rattus* to

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sandfly initiated *L. infantum* and their subsequent infectivity for sandflies. Failure to detect parasites in heart blood may be related to leishmanicidal activity of sera observed in some animal species (Rezai et al., 1975). The use of a combination of parasite detection and isolation methods may enhance chances of parasite recovery. Further, the sampling of dermal tissues, such as those located in the extremities may provide tentative indication of parasite accessibility to potential vectors.

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