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SCIENCE DIRECT
Microbes and Infection 8 (2006) 1691–1694
Microbes and Infection
www.elsevier.com/locate/micinf

Original article

# Experimental transmission of *Leishmania tropica* to hyraxes (*Procavia capensis*) by the bite of *Phlebotomus arabicus*

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Received 2 December 2005; accepted 30 January 2006
Available online 21 April 2006

**Abstract**

The ability of the sand fly *Phlebotomus* (*Adlerius*) *arabicus* to transmit *Leishmania tropica* was studied experimentally using hyraxes (*Procavia capensis*), natural reservoir hosts of the parasite. Sand flies became infected with *L. tropica* after feeding on a lesion of needle-inoculated hyrax. Moreover, *P. arabicus* fed with *L. tropica* promastigotes transmitted the parasite to hyraxes by bite during a second bloodmeal. Although the animals remained asymptomatic after infective sand fly bite, they were PCR positive and infectious for naive sand flies. We have thus demonstrated cyclical transmission of *L. tropica* by *P. arabicus* in hyraxes. This confirms experimentally the vectorial competence of *P.* (*Adlerius*) *arabicus*, and demonstrates that asymptomatic reservoir hosts are infectious to appropriate vectors.


*Keywords:* Asymptomatic reservoir host; Sand fly; Vector competence; Cutaneous leishmaniasis; Zoonosis

## 1. Introduction

Leishmaniases are diseases caused by protozoan parasites (Kinetoplastida: Trypanosomatidae), their clinical features varying from localized cutaneous lesion to generalized life-threatening visceral disease. Vectors of leishmaniases, females of phlebotomine sand flies (Diptera: Psychodidae), transmit the disease while taking a bloodmeal on a susceptible vertebrate host.

Leishmania species causing Old World cutaneous leishmaniases (CL) differ in their epidemiology. CL due to *Leishmania major* is a zoonosis, with rodents serving as reservoir hosts. On the other hand, CL due to *L. tropica* is usually regarded as an anthroponosis, although the parasite was isolated from several mammals other than man, e.g. black rats and dogs [1–3]. However, it is highly probable that these animals are not reservoir hosts, since the infection is extremely rare, and they might be considered 'victims' [4,5]. Nevertheless, while in hyperendemic situations man-to-man transmission of *L. tropica* is well ascertained, the occurrence of sporadic human cases in some foci suggests zoonotic transmission. A suspected reservoir host is the hyrax (*Procavia capensis*). *Leishmania* parasites were repeatedly isolated from hyraxes [6,7]; in some CL foci, hyraxes are numerous and peridomestic, and *L. tropica* was detected in their tissue by PCR [8].

We have recently isolated a strain of *L. tropica* from a hyrax, which, using ITS1-RFLP, is identical to human and vector isolates from the focus situated in the northern banks of the Sea of Galilee in Israel (Svobodová et al., unpublished). In this focus, a new sand fly species, *Phlebotomus* (*Adlerius*) *arabicus*, was identified as a vector of CL [8]. In this study, we confirm the vectorial status of this sand fly experimentally. We demonstrate transmission of *L. tropica* by bite of *P. arabicus* to hyraxes, thus proving the vectorial competence of this sand fly species and the potential of the hyrax to serve as reservoir host.

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doi:10.1016/j.micinf.2006.01.024

## 2. Materials and methods

### 2.1. Sand flies

The *P. arabicus* colony was established from gravid females caught in CDC light traps in September 2001 in the village Amnun, in Northern Galilee, Israel. The colony was maintained at 21–23 °C, 100% humidity, and 14/10 light/dark photoperiod. Adults had permanent access to cotton wool soaked by 50% honey as sugar source. Twice a week females were allowed to feed on anesthetized mice (ketamin/xylazin 150 mg/kg and 15 mg/kg, respectively).

### 2.2. Parasites

*L. tropica* isolate MPRO/IL/2003/LRC-L 1015 originates from a hyrax caught in Amnun and was typed at the Department of Parasitology, Haddassah Medical School, Jerusalem. Blood agar from defibrinated rabbit blood, supplemented with 40 μg/ml gentamicin, was used for cultivation. In golden hamsters the isolate produced lesions after inoculation of $10^6$–$10^7$ stationary-phase promastigotes subcutaneously. Parasites used for hyrax infection were isolated from anesthetized hamster by needle aspiration.

### 2.3. Animals

Three male hyraxes that were used were born in captivity in the Czech Republic; their ancestors originated from the Republic of South Africa. Prior to all experimental procedures, they were anesthetized with ketamin (10%, 0.5 ml i.m.). Hyrax no. 1 was inoculated subcutaneously at the hairy site of the nose using $10^7$ stationary-phase promastigotes (subculture 1) supplemented with promastigote secretory gel (PSG) to enhance virulence [9]. PSG was isolated from *P. arabicus* infected with the same *L. tropica* strain by opening longitudinally the gut of heavily infected females with fine entomological needles. The PSG plugs were pooled in 5 μl each and centrifuged in Eppendorf centrifuge at 3000 rpm 4 times while collecting supernatant. The amount in the inoculum corresponded to PSG isolated from one female sand fly.

### 2.4. Sand fly infection and transmission to hyraxes

Sand flies were infected by feeding through a chick-skin membrane on heat-inactivated rabbit blood containing $5 \times 10^5$/ml promastigotes. Unfed females were separated 1 day after feeding. The sugar source for fed females was 50% honey. Females 10–11 days after the infectious first feeding were allowed to feed again on two uninfected hyraxes (nos. 2 and 3). Immediately after the second feeding, fed females were separated. The sites of feeding on the animals were recorded. Females were then dissected, and their guts were checked microscopically for the presence of *Leishmania* promastigotes.

### 2.5. Xenodiagnosis

Five to eight weeks after inoculation of promastigotes (hyrax no. 1) or feeding of infected sand fly females (hyrax nos. 2 and 3), uninfected *P. arabicus* females were allowed to feed on the hyraxes. Unfed females were separated 1 day after feeding. The sugar source for fed females was 50% honey; those were dissected 3 or 8–9 days after bloodfeeding to monitor the infection before and after defecation. Guts were checked under the light microscope for the presence of promastigotes.

### 2.6. PCR diagnosis

Biopsies from the nose were taken from all the hyraxes eight weeks after infection. Different scissors and forceps were used to avoid cross contamination with parasite DNA. Samples were homogenized in 1.5 ml microtubes in 50 μl NET-50 buffer. DNA was isolated from the tissue homogenates using the DNeasy® Tissue Kit (Qiagen) according to manufacturer's instructions. For PCR detection of *Leishmania* two DNA regions were used. The ribosomal internal transcribed spacer region (ITS1) was amplified with *Leishmania*-specific primers LITSR and L5.8S as described [8,10]. The kDNA fragment of 120 bp, present at ca. 10 000 copies in each parasite, was amplified with primers JW11 and JW12 [18]. Each sample was tested in triplicate. PCR products were analyzed by electrophoresis in a 1% agarose gel stained with ethidium bromide.

## 3. Results

### 3.1. Infection of the inoculated hyrax

A lesion appeared at the site of inoculation of hyrax no. 1 one month after infection. The lesion of approximately $10 \times 7$ mm was dry, protuberant, and whitish in appearance (Fig. 1A). Sand flies fed on this hyrax five weeks after inoculation. Females preferentially fed on the nose and lips, few fed on the eyelids and none on the ears. The infection rate was 7.1% in females dissected before the complete digestion of the bloodmeal ($N = 42$), and 4.2% after defecation ($N = 142$). This proved that the *L. tropica* strain used is transmissible to *P. arabicus* after feeding on an inoculated experimental animal, and that the infection persists after complete digestion of the bloodmeal.

### 3.2. Transmission by bite of P. arabicus

Ten to eleven days after membrane feeding on blood containing promastigotes, females were allowed to refeed on hyrax nos. 2 and 3. Ten infected females fed on the upper part of the nose and upper lip of hyrax no. 2, and five fed on the nose of hyrax no. 3. Seven or eight weeks after infective bite, uninfected *P. arabicus* females were allowed to feed on the hyraxes (Fig. 1B). The results of this xenodiagnosis were positive for hyrax no. 2, since one of 30 females was harboring promastigotes in its gut before defecation, and three of 61 were found infected after defecation (days 8–9 after feeding).