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Original article

Experimental transmission of *Leishmania tropica* to hamsters and mice by the bite of *Phlebotomus sergenti*

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Abstract

Phlebotomus sergenti is a natural vector of *Leishmania tropica*. However, the ability of *P. sergenti* to transmit *L. tropica* by bite has not been proven experimentally yet. We have transmitted *L. tropica* to golden hamsters and BALB/c mice by the bite of *P. sergenti*. Sand flies and *Leishmania* both originated from an anthroponotic cutaneous leishmaniasis focus in Urfa, Turkey. *P. sergenti* females from a laboratory colony were infected by feeding on lesions of needle-inoculated hamsters or mice. Gravid females were allowed to refeed on uninfected hosts 9–15 d after the infective feeding. At the second feeding, some infected females took a full blood meal, while others only a partial one; some females failed to feed at all. The ability of infected females to take a blood meal did not correlate with the parasite transmissibility. In four BALB/c mice, lesions developed after 1–6 months. In two albino hamsters (*Mesocricetus auratus*), lesions developed 1 month after the infective feeding, and *Leishmania* could be reisolated from these sites. Another hamster did not develop a lesion; however, the feeding site and the adjacent ear were PCR positive 1 year after infective feeding. Our results show that dissemination to other parts of host body occurs in *L. tropica* after sand fly bite. Experimental transmission of the parasite confirms that *P. sergenti* is a natural vector of *L. tropica*.

Keywords: Cutaneous leishmaniasis; Transmission; Vector competence; BALB/c mouse; *Mesocricetus*

1. Introduction

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

Leishmania was first transmitted experimentally by the bite of an infected female sand fly in 1931, when *Phlebotomus argentipes* infected Chinese hamsters (*Cricetus griseus*) with *Leishmania donovani* [1]. Ten years later, *L. infantum*, another Old World species causing visceral leishmaniasis, was transmitted to Chinese hamsters by the bite of *P. chinensis*, and cutaneous *L. major* was transmitted to man by the bite of *P. papatasi* [2,3]. The transmission of two other Old World cutaneous *Leishmania* species, *L. tropica* and *L. aethiopica*, has not yet been experimentally demonstrated. However, transmission by bite is one of the basic criteria of evidence of a vector [4].

P. sergenti has been found in anthroponotic cutaneous leishmaniasis foci, namely Taza, Morocco, Tanant, Tunisia, Urfa, Turkey, Allepo, Syria, and Kabul, Afghanistan [5–9]. *L. tropica* was first isolated from this vector in Saudi Arabia [10]. *P. sergenti* is considered to be a natural vector, based on the identity of human and vector isolates [5,10], and on its susceptibility to *L. tropica* after artificial infection [11]. It was also demonstrated that *P. sergenti* feeds on humans in nature [12]. Until now, evidence of experimental transmission by bite was lacking. In this study, we demonstrate the transmission of *L. tropica* to golden hamsters and BALB/c mice by the bite of its specific vector, *P. sergenti*.

2. Materials and methods

2.1. Sand flies

The *P. sergenti* colony was established from gravid females caught in CDC light traps in August 1998 in Urfa,

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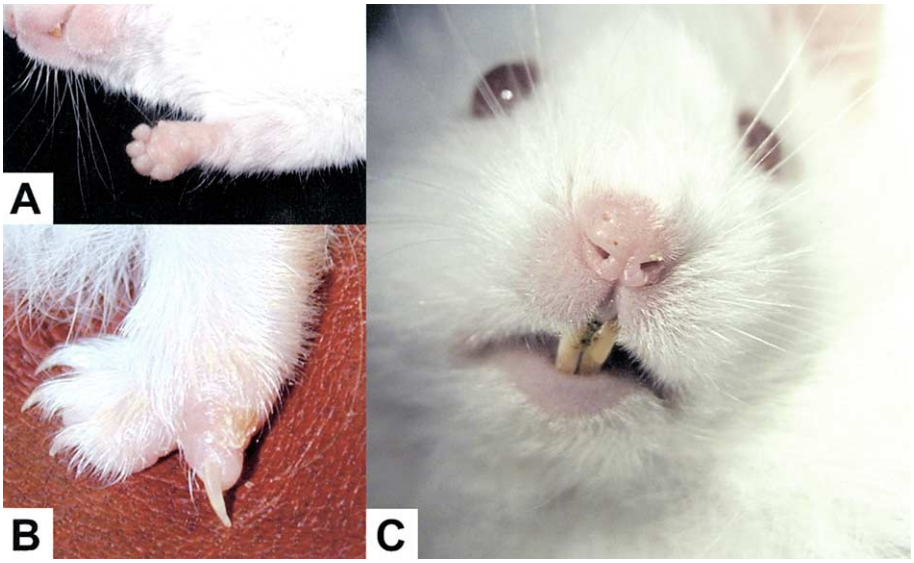

Fig. 1. Lesions caused by *L. tropica* in experimental hosts after feeding of the vector *P. sergenti*. (A) Forefoot of a BALB/c mouse, 6 months post-infection. (B) Hind foot finger of a golden hamster, 4 months post-infection. (C) Nose of a golden hamster, 1 month post-infection.

Turkey. The initial number of females was about 200. The colony was maintained at 26 °C, 100% humidity, and a 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 anaesthetized mice (ketamin/xylazin 150 and 15 mg/kg, respectively).

2.2. Parasites

L. tropica isolate MHOM/TR/1998/SU23 (zymodeme MON-53 and genotype IV) [13] originates from a patient with cutaneous leishmaniasis in Urfa, Turkey, and was typed in the laboratory of Prof. K.P. Chang, Chicago Medical School. Blood agar from defibrinated rabbit blood, supplemented with 40 µg/ml gentamycin, was used for cultivation. The isolates produced lesions in golden hamsters and BALB/c mice after inoculation of 10^6–10^7 stationary-phase promastigotes subcutaneously.

2.3. Sand fly infection

Sand flies were infected by feeding on hind feet lesions of needle-inoculated golden hamsters. 9–15 d after the infectious first feeding, females were allowed to feed again on an uninfected, anaesthetized laboratory rodent placed in the sand fly cage. Immediately after the second feeding, each fed female was separated, as well as females probing without taking a blood meal. The sites of feeding on the animal were recorded. Females were then dissected, and their guts were checked microscopically for the presence of *Leishmania* promastigotes. Three categories of the blood amount were distinguished: fully fed—the amount that would be taken by an uninfected female after uninterrupted feeding; partially fed—half or less blood than in the fully fed; and finally, no blood in the gut at all.

Rodents were checked every 2 weeks to monitor the lesion development. Lesions that appeared at the sites of sand fly feeding were biopsied using insulin syringe aspiration on anaesthetized animals, and cultivation was performed on blood agar. In those hosts that remained lesion-free for 1 year, PCR diagnosis was used.

2.4. PCR diagnosis

Tissues from the sites of sand fly feeding (feet, ear, piece of tail skin) as well as draining lymph nodes and eventually, adjacent ears, were taken from killed animals. Different scissors and forceps were used to avoid contamination with parasite DNA. Samples were homogenized in 1.5-ml microtubes in NET-50 buffer. DNA was isolated from the tissue homogenates using the DNeasy® Tissue Kit (Qiagen) according to manufacturer's instructions. PCR amplification with *Taq* polymerase (Promega) was performed using the JW11-JW12 primer pair [14], which amplifies a 120-bp fragment, present at ca. 10,000 copies in each parasite. The PCR conditions were: 4 min of initial denaturation at 94 °C, 35 cycles of 94 °C for 1 min, 58 °C for 30 s and 74 °C for 30 s, followed by a final extension at 74 °C for 10 min. Each sample was tested at least twice. The PCR products were analyzed by electrophoresis in a 1% agarose gel stained with ethidium bromide.

3. Results

Four BALB/c mice were subjected to feeding. One to 14 infected females took a second blood meal on individual mice. Three mice developed lesions on feet 6 months after sand fly feeding (Fig. 1A). The lesions did not ulcerate, and persisted for life. One mouse developed a lesion on the tail after 1 month; this small lesion disappeared after 6 months.

Six hamsters were used for the second feeding of infected females. One to six infected females fed on each animal. Two

of the hamsters developed multiple lesions 1 month after infectious feeding (Fig. 1B,C). The lesions did not ulcerate and persisted for life. Four of the hamsters never developed lesions or other signs of infection; however, one was PCR positive on the site of feeding and in the adjacent ear.

The amount of blood that the infected females were able to take varied. Eighteen females that took a second blood meal and transmitted the parasite (confirmed by lesion development or PCR) could be divided into three groups. Six took a full blood meal, while seven only a partial one. Five females failed to take any blood but were still able to transmit the parasite. Therefore, transmission of the parasite is not directly linked to the ability to take a blood meal.

To confirm the infectivity of *Leishmania* lesions developed after feeding of *P. sergenti* on model hosts, female sand flies were allowed to feed on the hind foot lesion of BALB/c mouse. Feeding was done 14 months after the infective sand fly feeding, and 8 months after the appearance of lesions. The dissection on day 8 after feeding revealed that 8/13 (62%) of the fed females acquired *Leishmania*. All had mature infection in thoracic midgut, and six of them were infected heavily.

4. Discussion

In our laboratory experiments, *P. sergenti* transmitted *L. tropica* to hamsters and mice. Female sand flies were infected by feeding on lesions. This method of infection mimics natural transmission. Membrane-feeding, frequently used to infect sand flies in the laboratory, results in higher infection rates, probably due to high infective doses. Furthermore, infection of sand fly species not naturally transmitting the disease is possible by membrane-feeding [15]. For example, *P. papatasi* can be infected with *L. tropica* after membrane-feeding, while no infection occurs after feeding on *L. tropica* lesions ([16], Svobodová, unpublished). Therefore, we preferred lesion feeding for infecting sand flies.

Laboratory mice were not the model host of choice for *L. tropica* for a long time, since they were believed to be unsusceptible to infection [17]. However, BALB/c mice were recently shown to develop lesions after inoculation of *L. tropica* strains from Urfa [18]. This study shows that BALB/c mice are also susceptible after transmission by bite, the natural route of infection. Transmissibility to BALB/c mice enables the use of an inbred model host for *L. tropica* infections.

Lesions developed in all four BALB/c mice after feeding of infected sand flies, while in golden hamsters, only two out of six developed lesions, probably due to the differences in genetic background of those outbred animals. A third hamster did not develop lesions, but parasites were present not only in the feeding site but also in the adjacent ear. Dissemination from the inoculation site to distant parts of the body was proven for *L. major* in mice [14]. Our results show that *L. tropica* is able to disseminate in the host as well, and that dissemination occurs after natural transmission by bite.

Parasites obstruct the anterior part of the digestive tract by forming a plug, consisting of promastigotes and secreted proteophosphoglycan gel [19,20]. *Leishmania* also damage the stomodeal valve by secreting chitinase [21]. Infected sand flies have difficulty in taking blood, which results in increased probing [22]. Damaged feeding mechanism or the presence of eggs in the abdomen might influence the volume of the second feeding. In our study, one third of transmitting females were able to take a full blood meal, while a third failed to feed at all; both groups effectively transmitted the parasite. Therefore, it is not necessary for the parasite to either allow blood taking or, on the contrary, to block the blood flow, as transmission occurs in both cases. However, multiple lesions may develop after feeding attempts of females that are unable to take blood [22], and this might enhance the subsequent transmission to parasite-free vectors.

In this study, we demonstrate successful transmission of *L. tropica* to golden hamsters and BALB/c mice by the bite of *P. sergenti*, thus confirming experimentally that *P. sergenti* is a natural vector of *L. tropica*.

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