Cutaneous leishmaniasis in Sri Lanka: a study of possible animal reservoirs

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Summary
Background: Cutaneous leishmaniasis (CL) has been detected with increasing frequency in Sri Lanka in recent years. Leishmania donovani has been identified as the causative agent, but no information is available on vector(s) or reservoir(s). In this paper we present data on the screening of possible reservoirs for evidence of infection.

Methods: Patients with clinically suggestive CL referred from dermatology clinics for a confirmatory diagnosis were examined parasitologically and by PCR. There were no immunocompromised patients and none had any visceralizing symptoms. Pet dogs and rodents from areas where the patients were diagnosed were similarly examined for infection.

Results: The disease was confirmed in 86 of 116 patients. All positive patients were from rural areas of the country, closely associated with scrub jungles. Of the 151 dogs examined, two showed Leishmania amastigotes in Giemsa-stained smears, one in the skin and one in peripheral blood. None of the 47 rodents screened showed any evidence of Leishmania infection.

Conclusions: The evidence gathered shows that in Sri Lanka the disease is restricted to persons in the hinterland areas, with a possibility of it being a zoonosis. The detection of Leishmania amastigotes in two dogs is, however, not sufficient to incriminate them as reservoirs. More studies are needed for evidence of reservoir(s) and identification of behavior of the vector species in order to explain the atypical presentation of L. donovani in Sri Lanka.

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Introduction

Leishmaniasis is a complex of diseases caused by different species and subspecies of the protozoan flagellate Leishmania. Most infections are zoonotic with a wide variety of animal hosts including rodents, with canids acting as reservoirs and humans as an accidental host.1
The zoonotic reservoir is well adapted to the parasite and thus develops a mild form of the disease. Though originally associated with forested areas, the transmission of leishmaniasis has now adapted to a domestic environment in some localities due to deforestation and urbanization.

The infection in humans appears in one of three main clinical forms viz. visceral, mucocutaneous, and cutaneous. Visceral leishmaniasis (VL) is the most severe form of the disease, which if left untreated has a mortality rate of almost 100%. Mucocutaneous leishmaniasis develops in a small percentage of persons infected with *Leishmania braziliensis* in Latin America. The cutaneous form of the disease is the most common and accounts for nearly 50—75% of all new cases that appear worldwide. The most common form of CL is the self-healing ulcer, which appears at the site of the sandfly bite.

The cutaneous form of the disease is now endemic in Sri Lanka, and the causative organism has been identified as *Leishmania donovani*. Karunaweera et al. have identified *L. donovani* zymodeme MON-37 as the causative species of CL in Sri Lanka. *L. donovani* is commonly considered to be responsible for VL. There are reports, however, of *L. donovani* causing CL from several other countries.

There is a paucity of information in respect of vector(s) and reservoir hosts, if any, in Sri Lanka. Further it is not known whether the disease in Sri Lanka is a zoonosis. We have previously reported the clinical aspects and geographical distribution of the disease in this country, and in this paper we present data that lend support to the possibility of the disease being a zoonosis in Sri Lanka.

**Materials and methods**

**Patients**

The patients examined in this study were those referred to the Department of Parasitology by the consultant dermatologists in government hospitals for a confirmatory diagnosis of CL. A total of 116 patients with no history of foreign travel were examined. A detailed clinical and social history was obtained using a structured questionnaire, and a complete clinical examination was performed on each patient to exclude any history of immunosuppressive illnesses. Skin lesions were examined and samples were obtained as previously reported. Ethical clearance for the study was granted by the Research and Higher Degrees Committee of the Faculty of Medicine, University of Peradeniya.

**Animals**

The animals examined were domestic dogs and rodents from areas where the CL patients were diagnosed. In this study a total of 15 villages were covered in the three provinces, Central, North Western, and North Central, and sample collections were carried out simultaneously with patient studies.

For the collection of samples from dogs, house visits were made with the veterinary surgeons in the target areas by prior arrangement. A brief history of the animal was obtained from the owner before examination. Needle biopsies from skin and from ulcers, if any, were obtained for smears and PCR. Venous blood was taken into a sterile vial containing 3.8% sodium citrate and buffy coat separated. The samples (buffy coat and skin) for PCR were stored at −20°C.

Field rodents were trapped live using wire mesh (1 × 1 cm) traps (60 × 25 × 25 cm) with trigger-released drop-down doors. The traps were set in and around houses, the peridomestic environment, and in the surrounding crops. Trappings were done over a period of one year at triweekly intervals and at each visit 15—20 traps were set. Trapped rodents were transported to the laboratory for examination.

The rodents were identified using the morphological characteristics described by Philips. They were euthanized using chloroform and examined carefully for evidence of any cutaneous lesions. Each sacrificed animal was scrubbed well with soap and running tap water using a brush. Skin, blood, liver, and spleen samples were obtained from each, using sterile instruments after heat cautery, for smears, culture, and PCR.

Permission to trap and transport rodents was obtained from the Department of Wild Life and Forestry, Sri Lanka.

**Parasitological diagnosis**

All impression smears were stained with 10% Giemsa and were examined under oil immersion (×100). Evans’-modified Toby’s medium was used for in vitro culture with 1% glucose in normal saline as the liquid overlay. Culture tubes were stored in a dark container at 24 ± 1°C in a temperature controlled room and were examined from day 3 post-inoculation. Cultures were examined for a maximum period of four weeks before discarding them as negative.

**Polymerase chain reaction (PCR)**

DNA was extracted from frozen tissue samples and buffy coats using DNAzole (GIBCO BRL, Life Technologies, USA) according to the manufacturer’s protocol. PCR was performed using a set of primers (5’ CGGCTTGCACCACCATGGTG 3’; 5’ ACATCCCTGCCCACATAGC 3’) specific for all old world *Leishmania spp.*, which amplified a 260-bp region in the genomic DNA. PCR amplifications were carried out in a thermocycler (Thermolyne Amplitron 11) with a heated lid and auto-chill facility. A standard protocol (40 cycles, 30 s at 94°C, 30 s at 56°C, and 20 s at 72°C) was followed. PCR products were separated on 1% agarose gel stained with ethidium bromide.

**Results**

**Patients**

Of the 116 patients, 86 (74.1%) were diagnosed as positive for CL based on one or more of the diagnostic methods employed. The male:female ratio of positive patients was 1.4:1 (50 males and 36 females) and their ages ranged from 3 to 70 years (Table 1). The majority of the patients were school children (Table 2).

Positive patients came from 12 of 24 administrative districts representing eight of nine provinces of the country. All patients had behavior associated with natural reserves or...
scrub jungles. This was clearly seen in 47 of 86 (54.7%) patients who were clustered in two districts — Kurunegala and Matale — in the Central province of the country. A spot map drawn using geographic information system (GIS) software showed that these patients were clustered in areas in close proximity to natural reserves and scrub jungles. We observed the houses of these patients, barring a few, to be situated either in or on the fringes of these natural reserves (Figure 1).

**Table 1** Age and gender distribution of cutaneous leishmaniasis-positive patients included in the study (*N* = 86)

<table>
<thead>
<tr>
<th>Age</th>
<th>f (%)</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–10</td>
<td>12 (14.0)</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>11–20</td>
<td>18 (20.9)</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>21–30</td>
<td>14 (16.3)</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>31–40</td>
<td>23 (26.7)</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>41–50</td>
<td>7 (8.1)</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>51–60</td>
<td>8 (9.3)</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>61–70</td>
<td>4 (4.7)</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

**Table 2** Occupations of the 86 cutaneous leishmaniasis patients included in the study

<table>
<thead>
<tr>
<th>Occupation</th>
<th><em>n</em></th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-school children</td>
<td>3</td>
<td>3.5</td>
</tr>
<tr>
<td>Students</td>
<td>27</td>
<td>31.4</td>
</tr>
<tr>
<td>Housewives</td>
<td>12</td>
<td>14.0</td>
</tr>
<tr>
<td>Farmers (agriculture)</td>
<td>12</td>
<td>14.0</td>
</tr>
<tr>
<td>Service personnel</td>
<td>18</td>
<td>20.9</td>
</tr>
<tr>
<td>Other&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14</td>
<td>16.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Four teachers, four drivers, five laborers, and a carpenter.

**Table 3** Geographical distribution of dogs and rodents examined in the study

<table>
<thead>
<tr>
<th>Province</th>
<th>Number of animals examined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dogs</td>
</tr>
<tr>
<td>Central</td>
<td>82</td>
</tr>
<tr>
<td>North Western</td>
<td>45</td>
</tr>
<tr>
<td>North Central</td>
<td>24</td>
</tr>
</tbody>
</table>

Dogs

A total of 151 dogs were examined (Table 3), and of these, two had skin ulcers that were suspicious. Leishmania amastigotes were seen in a blood smear from a single dog from Naula and in a skin impression smear from a single dog from Dambulla in the district of Matale. The organisms were morphologically similar to those in patients, but pseudocysts were not encountered. However, PCR on buffy coat DNA from both animals failed to show any positive bands. None of the

![Figure 1](image-url)  
*Figure 1* Spot map showing clustering of positive patients in the districts of Matale and Kurunegala.
other dogs were positive for Leishmania either by culture, smear, or PCR.

Rodents

Forty-seven rodents were examined during the study period (Table 3). Of these, one was a Ceylon gerbil (*Tatera indica indica*), 12 were bandicoots (*Bandicota indica indica*), and 34 were black rats (*Rattus rattus*). Four bandicoots had scars at the base of the tail. One *Rattus rattus* had an ulcer on the neck, whilst another had multiple ulcers on the ear. However, none was positive for Leishmania by any of the diagnostic methods used.

Discussion

CL is now endemic in Sri Lanka.7,8 In our study the disease was diagnosed in patients from 12 districts in eight of nine provinces, Uva province being the exception.7 A few positive patients, however, have been reported by other workers from Uva province,5 and thus the disease may now be considered to be present in all provinces of the country.

All these studies, however, raise the question of whether the disease is a recent introduction into the country, or whether there has merely been an increase in recognition of an existing disease or an emergence of an infection that already existed, due to human encroachment into the sylvatic environment.

Introduction of the disease into the country is a possibility, perhaps coming from neighboring India, the Middle East, and Africa due to increased travel for employment, trade, and leisure, although no strong evidence of this is available. Naotunne et al.15 have already remarked on this, having detected the disease in returnees from the Middle East and Africa.

An increase in the population and migration of refugees has resulted in the establishment of new settlements, thereby intruding into the natural sylvatic environment. In Pakistan it has been reported that the civil war situation increased the incidence of CL due to the associated deterioration of the infrastructure and increased migration levels.16 There are records on epidemics of localized CL being associated with mass population movement, settling in previously uninhabited areas,17 deforestation, road construction, and war.10

All our patients were from the rural areas in the dry and intermediate climatic zones of the country.7 An interesting feature was that only one member in a family was affected and that other family members as well as neighbors were free of clinical infection. Further, in each village the number of patients was restricted to a few, and some distance separated their houses from each other. All the affected people in these houses had behavior associated with scrub jungles. These observations led us to believe that anthropotic transmission may be a rarity and that the disease is a zoonosis. In most endemic countries CL is considered to be a zoonosis with canids and rodents acting as reservoirs.18 However, rarity of infection in animals is known19 and presents a major problem in the search for reservoirs.

Although we could examine only 47 rodents during the twelve-month study period, a thorough investigation was performed on each rodent for evidence of any leishmanial infection. Ulcers were found to be common in the tail and ear but none were positive for infection. However we did detect Leishmania organisms in two dogs; in one in the skin and in the other in blood. There are records of Leishmania being detected in dogs from Sri Lanka in blood smears and by serology.5 Canids have been shown to be reservoir hosts for many different *Leishmania* species.20

Vector species and their behavior is an important factor that influences the clinical presentation of leishmaniasis in humans.21 Lewis22 identified two species of Phlebotomus in Sri Lanka (*Phlebotomus argentipes* and *Phlebotomus stantonii*) and found *P. argentipes* to be morphologically different to that in India, our closest neighbor. *P. argentipes* in the lowlands of Sri Lanka are weakly anthropophagic,23 whilst those found in the central highlands are known to be more anthropophagic.24 The latter authors were of the opinion that the allopatric populations are predominantly zoophilic in lowlands and more anthropophilic in the highlands.

It needs to be pointed out that CL was encountered in the dry and intermediate zones in the low country areas of Sri Lanka.7 No patients were diagnosed from high altitude areas. A zoophilic *P. argentipes* thus could very well fit to complete the triangle in a zoonotic transmission for this dermatrophiic *L. donovani* in Sri Lanka. Similarly Sharma et al.10 were of the opinion that the CL due to *L. donovani* in the new endemic focus of Himachal Pradesh in India could be the result of a change in vector species. Although they found no evidence, they observed a preponderance of *Phlebotomus longiductus* in this focus.

The detection of Leishmania amastigotes in dogs along with the presence of a zoophilic *P. argentipes* in the lowland areas where human infections were detected, lends support to CL being a zoonotic disease in Sri Lanka.

A limitation of this study is the number of animals examined, which may not be adequate to incriminate dogs as a reservoir. Nevertheless our findings support the earlier observations regarding the presence of leishmaniasis in dogs in Sri Lanka. We strongly believe this report will encourage further studies to confirm dogs as a possible zoonotic reservoir for Leishmania.

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Conflict of interest: No conflict of interest to declare.

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