

## Short Report: Epidemiology of Imported Cutaneous Leishmaniasis at the Hospital for Tropical Diseases, London, United Kingdom: Use of Polymerase Chain Reaction to Identify the Species

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**Abstract.** This study reviewed all patients diagnosed with imported cutaneous leishmaniasis (CL) at the Hospital for Tropical Diseases in London, United Kingdom, over an 11-year period. Diagnostic and epidemiologic information was collected prospectively for all patients with imported CL to this hospital during 1998–2009. A total of 223 patients were given a diagnosis of CL. Ninety patients were diagnosed with Old World CL, which was caused most commonly by *Leishmania donovani* complex (n = 20). A total of 71% were tourists to the Mediterranean region, 36% were migrants or visiting friends and relatives, and 17% were soldiers. One hundred thirty-three patients were given a diagnosis of New World CL. The *Leishmania* subgenus *Viannia* caused 97 of these cases; 44% of these were in backpackers and 29% were in soldiers. Polymerase chain reaction was more sensitive and faster for detecting *Leishmania* DNA (86% for Old World CL and 96% for New World CL) than culture. This is the largest study of imported leishmaniasis, and demonstrates that tourists to the Mediterranean and backpackers in Central and South America are at risk for this disease.

Cutaneous leishmaniasis (CL) is the commonest form of leishmaniasis and has an estimated 1.5 million infections annually worldwide.<sup>1</sup> This disease predominantly occurs in persons living in disease-endemic countries but also is an increasing problem in travelers. The Hospital for Tropical Diseases in London provides tertiary level clinical care for patients with imported CL, and hosts the United Kingdom Health Protection Agency Reference Laboratory for Parasitology.

Cutaneous leishmaniasis is categorized into Old World CL and New World CL. Differentiation is important because mucosal disease (ML) is caused by New World CL, specifically *Leishmania (Viannia)* spp. and requires systemic treatment.<sup>2</sup> Skin lesions are classically ulcerated, but may be nodular or keratotic.

Endemic CL is caused by *L. infantum* in Spain and Portugal;<sup>3</sup> *L. infantum*, *L. major*, and *L. tropica* in Cyprus and Turkey;<sup>4,5</sup> by *L. major* and *L. tropica* in Iran, India, and Pakistan;<sup>6–8</sup> and by *L. aethiopicum*, *L. major*, and *L. infantum* in Ethiopia and Saharan Africa.<sup>9–11</sup> *L. (Viannia)* spp. is the predominant cause of New World throughout Central and South America.<sup>12</sup>

Non-healing skin lesions in a patient with an appropriate travel history referred to the Hospital for Tropical Diseases undergo a skin biopsy, of which a sample is examined for *Leishmania* spp. by microscopy, histologic analysis, and culture. The sensitivity of these investigations ranges from 33% to 74%.<sup>13,14</sup> The polymerase chain reaction (PCR), which has been used to detect *Leishmania* DNA, has improved diagnostic sensitivity to 95%.<sup>13</sup> The Hospital for Tropical Diseases has used PCR for diagnostic DNA detection in all suspected cases of CL since 1996.

The purpose of this study was to use the large comprehensive Hospital for Tropical Diseases clinical database of all laboratory-proven cases of leishmaniasis over an 11-year period (1998–2009). Our main objective was to report the epidemiology of imported CL, correlations of the type of traveler with the country of travel, and the species of *Leishmania* acquired.

We looked for regional correlations between species known to cause local endemic disease and those species acquired by travelers (Tables 2 and 3).

Clinical details were obtained from case notes when the laboratory recorded a positive result for *Leishmania* spp. Data obtained included type of traveler, country of travel, time to referral, diagnostic tests performed, and *Leishmania* species.

The laboratory received fresh and fixed specimens. A dab preparation was made from the fresh specimens and stained with Giemsa or modified Rapid Field stain and inspected microscopically for amastigotes. Fresh material was also inoculated into modified Novy-Nicolle-McNeil medium and growth was checked weekly for 14–21 days. The time from the sample being received by the laboratory to confirming the diagnosis of infection with *Leishmania* spp. by any modality was recorded. *Leishmania* DNA was extracted, and amplification was attempted by using PCR on all samples from cases of suspected leishmaniasis.

All extractions were performed by using the Qiagen Tissue and Blood Mini Kit (Qiagen, Crawley, United Kingdom). Histologic sections were de-waxed by using xylene and absolute ethanol before extraction. During the study, primer sets were changed and the repertoire increased, as shown in the algorithm in Figure 1, because more species-specific primers became available.

The most sensitive PCR (nested) used in species identification for most cases uses kinetoplast minicircle DNA.<sup>15,16</sup> The PCR products were subjected to electrophoresis on agarose gels containing ethidium bromide, and diagnosis and species identification was performed by comparison with a molecular mass standard and amplified DNA from control strains.

A total of 223 patients were given a diagnosis of CL during 1998–2009. Of these patients, 133 were given a diagnosis of New World CL and 90 were given a diagnosis of Old World CL (Table 1). More males than females were infected (67% with Old World CL versus 67% with New World CL 70%), and the median age was 26.5 years. The median time from lesion onset to definitive diagnosis was five months for patients with Old World CL (range = < 1–35 months) and two months for patients with New World CL (range = < 1–24 months). Tourists (32%) and migrants either entering

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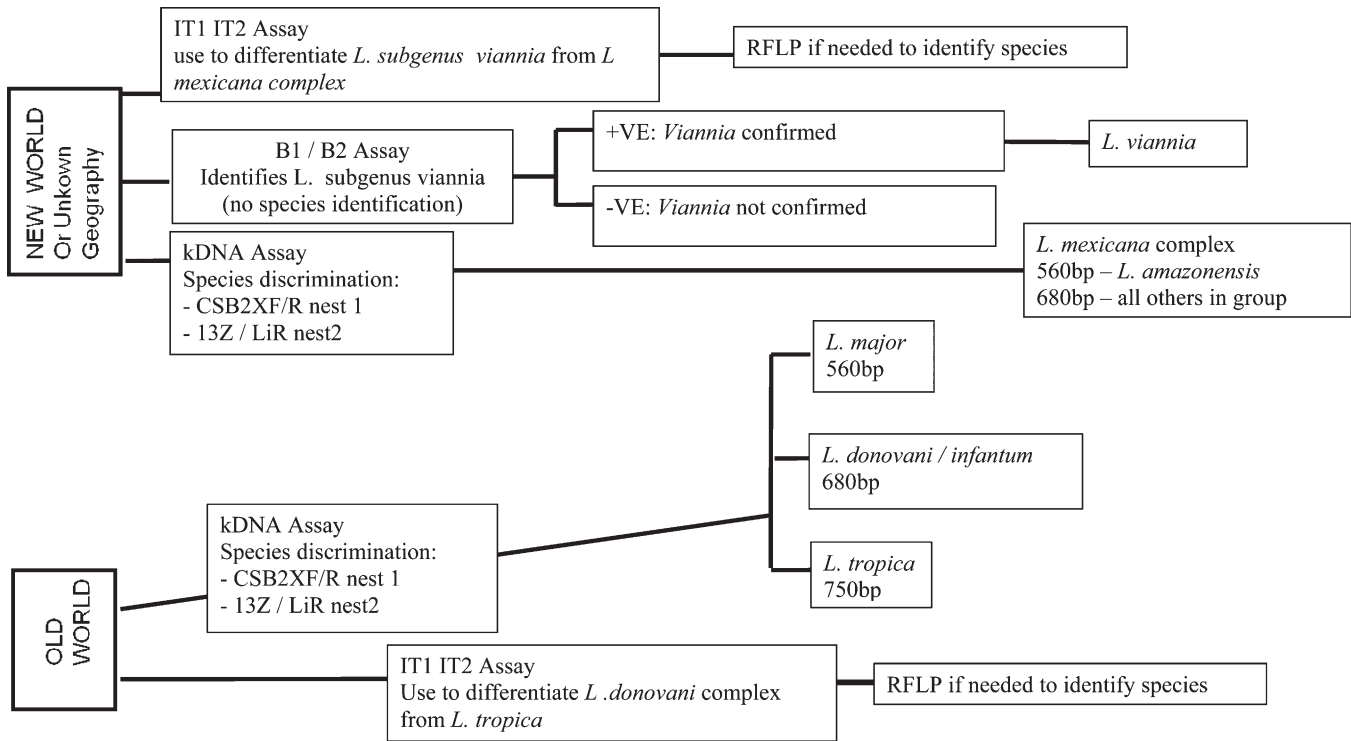


FIGURE 1. Algorithm for diagnosis of *Leishmania* infection by polymerase chain reaction, Hospital for Tropical Diseases, London, United Kingdom.

the United Kingdom or visiting friends and relatives in a disease-endemic country (36%) predominated in the Old World CL group. Twenty-five tourists acquired Old World CL in the Mediterranean basin, particularly in Spain (11 of 25, 44%). All military personnel acquired their disease in Iraq or Afghanistan.

New World CL occurred mainly in travelers on backpacking trips (44%) and in soldiers (29%) performing jungle train-

ing in Belize. There were 11 cases of ML in patients with New World CL; all were caused by *L. (Viannia) spp.* (Table 1).

Countries of acquisition of Old World CL are shown in Table 2. The commonest region was the Mediterranean; this region had 20 cases caused by the *L. donovani* complex (the Hospital for Tropical Diseases PCR cannot distinguish between the DNA of *L. donovani* and *L. infantum*). *Leishmania* was the predominant species and caused Old World CL in Afghanistan, Pakistan, and Iraq (n = 18). *Leishmania major* caused Old World CL in Pakistan, Iraq, Afghanistan, and Africa (n = 19).

Countries of acquisition for New World CL are shown in Table 3. Of 133 cases, country of acquisition was available for 127 travelers. Eighty-one had traveled to Central America and 46 to South America. *Leishmania (Viannia) spp.* caused 97 (86%) cases of New World CL and was associated with travel to Central America (n = 56) and South America (n = 41). *Leishmania mexicana* was detected in 26 (32%) cases associated with Central America, particularly Belize; only one case was acquired in South America. There were three cases of infection with *L. amazonensis*, all of which were acquired in South America.

For Old World CL, sensitivity was 86% for the PCR, 52% for culture, and 62% for microscopy. The sensitivity of tests for NWCL was 96% for PCR, 68% for culture and 77% for microscopy (Table 4). Because positive results for histologic samples were not fully recorded in the database, these data are not reported. The mean time to a positive diagnosis by PCR was 8 days and to any result either positive or negative by culture was 11.6 days (n = 134) (P < 0.005, by paired two-tailed t-test).

TABLE 1  
Demographic characteristics of study population, Hospital for Tropical Diseases, London, United Kingdom\*

| Characteristic                             | Old World CL† | Range or % | New World CL† | Range or % |
|--|---------------|------------|---------------|------------|
| Total                                      | 90            |            | 133           |            |
| Male sex                                   | 60 (67)       |            | 93 (70)       |            |
| Median age at presentation, years          | 27            | 3-86       | 26            | 2-80       |
| HIV infected‡                              | 0/11          |            | 0/21          |            |
| Median time to presentation, months        | 5             | < 1-35     | 2             | < 1-24     |
| Mucosal involvement                        | 0/90          |            | 11/133        |            |
| Type of traveler                           |               |            |               |            |
| Visiting friends and relatives/new entrant | 33            | 36         | 2             | 1.5        |
| Tourist                                    | 29            | 32         | 7             | 5          |
| Soldier/military                           | 15            | 17         | 39            | 29         |
| Backpacker/NGO worker                      | 3             | 3          | 59            | 44         |
| Other (business, missionary)               | 8             | 9          | 24            | 18         |

\* CL = cutaneous leishmaniasis; HIV = human immunodeficiency virus; NGO = non-government organization.  
 † Values are no. (%) unless otherwise indicated.  
 ‡ Thirty-two persons were tested for HIV.

TABLE 2

Epidemiologic distribution of Old World *Leishmania* spp. causing cutaneous leishmaniasis, Hospital for Tropical Diseases, London, United Kingdom

| Location   | <i>L. donovani</i> | <i>L. tropica</i> | <i>L. major</i> | <i>L. aethiops</i> | Not speciated | Total per country |
|--|--------------------|-------------------|-----------------|--------------------|---------------|-------------------|
| Mediterranean (European countries bordering the Mediterranean Sea including Cyprus, Greece, Majorca, Malta and Gozo, Portugal, Spain and the Balearic Islands, and Sicily) | 20                 | 1                 | 0               | 0                  | 4             | 25                |
| Near East (Jordan, Turkey, Syria, Yemen, and Israel)   | 4                  | 3                 | 5               | 0                  | 5             | 17                |
| Pakistan   | 2                  | 6                 | 1               | 0                  | 1             | 10                |
| Afghanistan  | 2                  | 9                 | 2               | 0                  | 6             | 19                |
| Africa (Continental Africa including all countries bordering the Sahara)   | 0                  | 0                 | 3               | 1                  | 2             | 6                 |
| India  | 0                  | 0                 | 0               | 0                  | 1             | 1                 |
| Iraq   | 0                  | 0                 | 8               | 0                  | 4             | 12                |
| Total  | 28                 | 19                | 19              | 1                  | 23            |                   |

This study is the largest case series of imported CL from a tertiary referral center in Europe. Data clearly show that different Old World CL *Leishmania* species in travelers are found in distinct geographic areas. Endemic disease patterns correlate closely with travel-acquired CL, and identical species cause disease in each region.

The large numbers of *L. donovani* complex cases acquired in Spain and the Balearic Islands is probably associated with increasing numbers of British tourists traveling to Spain. A study from our institute (n = 42 over 3 years) showed that 40% of patients with CL were tourists.<sup>17</sup> Other centers have reported similar trends for Old World CL and New World CL.<sup>18,19</sup> Lawn and others at the Hospital for Tropical Diseases reported increasing numbers of imported cases of New World CL during 1999–2003 (79 CL and 6 ML),<sup>20</sup> which reflected increasing numbers of travelers at risk for acquiring CL rather than an overall increase in the disease. Infections acquired in the Near East/Middle East and Africa were in military personnel and persons visiting friends and relatives. Soldiers have been in Iraq and Afghanistan since 2001. The increase in cases in persons visiting friends and relatives may be caused by increasing numbers of migrants to the United Kingdom; soldiers caused the predominance of males in the Old World CL group.

The large number of case of New World CL seen in travelers was unexpected. A study in the Netherlands reported 78 patients seen over 10 years, of whom 78% had New World CL, but did not give detailed reasons for travel.<sup>19</sup> In our study, most cases of New World CL from this region were military personnel, (from the United Kingdom Government's jungle training facility in Belize) and adventure tourists. Only two (1.5%) of our cases of New World CL were in immigrants/ persons visiting friends and relatives compared with 36% in the Old World CL group.

As shown in other studies,<sup>5,15,21</sup> detection of *Leishmania* DNA by using PCR is highly sensitive for the diagnosis of CL and identifies species. Specificity of PCR is also high. Previous work from our laboratory on smaller numbers of samples has shown a sensitivity of ≈100% for detection of *L. (Viannia)* spp. DNA by using PCR.<sup>17,20,22</sup> This larger data set showed a definitive sensitivity of PCR at the Hospital for Tropical Diseases of 86% for Old World CL and 96% for New World CL. Diagnosis by using PCR significantly reduces the time to obtain a definitive diagnosis and is the only technique yielding a species that can be used on fixed specimens.

Only 32 patients were tested for infection with human immunodeficiency virus; all had negative results. This finding contrasts with that for visceral leishmaniasis, for which infection with this virus is an established risk factor. Our study was restricted to demographic details that had been recorded in the case notes for most cases. Therefore, we were unable to obtain detailed information for some patients.

Polymerase chain reaction was performed for all specimens, but the date at which a negative PCR result is final was not routinely recorded. Therefore, we could only calculate the diagnosis times for cases with positive results, which led to potential bias. We also only included patients seen in our own unit. Therefore, our data are likely not to be completely representative of all cases of CL in the United Kingdom.

Our study is the largest case series of imported CL reported in the literature. We have identified a range of different *Leishmania* species in travelers, associated with particular demographic groups, who all acquired locally endemic disease. The use of PCR to detect *Leishmania* DNA has improved the sensitivity and specificity of laboratory diagnosis. Use of this technique to identify species enables the treating clinician to select the most appropriate therapy and significantly reduces time to definitive diagnosis.

TABLE 3

Epidemiologic distribution of New World *Leishmania* spp. causing cutaneous leishmaniasis, Hospital for Tropical Diseases, London, United Kingdom

| Location  | <i>L. (Viannia)</i> spp. | <i>L. mexicana</i> | <i>L. amazonensis</i> | Total per country |
|---|--------------------------|--------------------|-----------------------|-------------------|
| Belize  | 38                       | 18                 | 0                     | 56                |
| Bolivia   | 19                       | 1                  | 0                     | 20                |
| Peru  | 11                       | 0                  | 0                     | 11                |
| Central America (excluding Belize and the northern border of Mexico to the northern border of Colombia) | 18                       | 7                  | 0                     | 25                |
| South America (excluding Bolivia or Peru)   | 11                       | 1                  | 3                     | 15                |
| Total   | 97                       | 27                 | 3                     | 127               |

TABLE 4

Sensitivity of diagnostic tests used to identify *Leishmania* spp. causing cutaneous leishmaniasis, Hospital for Tropical Diseases, London, United Kingdom\*

| Test       | Old World CL, no. positive/<br>no. tested (%) | New World CL, no. positive/<br>no. tested (%) |
|------------|---|---|
| Microscopy | 38/61 (62)                                    | 90/117 (77)                                   |
| Culture    | 33/63 (52)                                    | 78/114 (68)                                   |
| PCR        | 67/78 (86)                                    | 124/129 (96)                                  |

\* CL = cutaneous leishmaniasis; PCR = polymerase chain reaction. Sensitivities reported are for the Hospital for Tropical Diseases PCR overall for New World CL and Old World CL. The primer set increased during the study (Figure 1), but individual sensitivities for each primer set have not been calculated. There were no patients from whom samples were culture positive and PCR negative.

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