Cutaneous leishmaniasis : new developments in diagnosis and treatment evaluation
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Citation for published version (APA):

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Download date: 03 Jan 2019
Epidemiology of cutaneous leishmaniasis in Suriname;

A study performed in 2006

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Submitted
Epidemiology of CL in Suriname

Summary

Cutaneous leishmaniasis (CL) is a widespread disease in Suriname caused by *Leishmania Viannia guyanensis*. It is suggested that also other *Leishmania* species are responsible for CL and that incidence is increasing. This study aimed to identify *Leishmania* species causing disease and to estimate the annual detection rate of CL in Suriname in 2006. In Paramaribo, 152 patients were registered, from which 33 could be tested in two PCR-RFLP methods. Twenty-seven patients were infected with *L. (V.) guyanensis* (complex), one with *L. (V.) lainsoni* and one with *L. (Leishmania) amazonensis*. In the hinterland 162 CL suspected patients were registered by means of questionnaires, from which 24 out of 27 were positive in PCR-RFLP: 88.9% (95% CI 77.1% - 100%). With extrapolation an annual detection rate was calculated of 5.32 to 6.13 CL patients per 1,000 inhabitants for the hinterland and 0.64 to 0.74 patients per 1,000 inhabitants for the whole country.
Introduction

Leishmaniasis is a vector-borne disease caused by the obligate intracellular protozoan *Leishmania* parasites of vertebrate hosts, including humans. The disease is prevalent throughout the world in 88 countries, with an overall prevalence of 12 million people, causing a burden estimated at 946,000 disability adjusted life years (DALY) for men and 1,410,000 for women.\(^1\) In Suriname, an endemic country in the northern part of South America cutaneous leishmaniasis (CL) is widespread in the primary rain forest and mainly affects people during the rainy seasons. It was already described in 1911 and is locally known as *Boschyaws* or *Boessie-Yassi*.\(^2\) CL was considered a minor health problem compared to malaria in Suriname, thus few reports have been written on incidence, identity of parasite, vector and host reservoir.\(^3\)

Last estimations of the annual incidence of CL in Suriname were made between 1979 and 1985; 4.9 cases per 1,000 inhabitants for the forested hinterland and 0.66 per 1,000 inhabitants for the whole country.\(^4\) The sandfly *Phlebotomus anduzei* was described as vector\(^5\), currently believed to be *Lutzomyia umbratilis*.\(^3\) The two-toed sloth (*Choleopus didactylus*), the anteater (*Tamandua tetradactyla*) and several species of marsupials and rodents are assumed to be reservoirs of *Leishmania* in Suriname.\(^6\) So far, only *Leishmania (V.) guyanensis* is described as causative organism of human CL in Suriname.\(^7\) One case with visceral leishmaniasis has been reported in 1953\(^8\), but in that case the parasite was not analytically identified, and could have been mistaken for *Histoplasma* spp.\(^9\) However, the Dermatological clinics in the country are at present encountering patients with more disseminated forms of CL and even mucocutaneous involvement with variable responses to treatment, suggesting the presence of other infecting *Leishmania* species.\(^4\)

Species identification is essential, as the prognosis of CL varies with and choice of treatment depends on the causative *Leishmania* species.\(^10\) Multilocus enzyme electrophoresis is the gold standard for identification of *Leishmania* (sub-) species and represents the basis of current taxonomy.\(^11\) However, PCR-Restriction Fragment Length Polymorphism (RFLP) represents a good alternative, circumventing the need for parasite isolation and cultivation.\(^12-14\) PCR-RFLPs are often directly applicable to clinical samples,
less technically demanding and allow high throughput analyses. The first objective of this study was the identification of infecting *Leishmania* parasites in Suriname. Therefore, two PCR-RFLP methods were performed on skin biopsies of CL suspected patients who were prospectively included in Paramaribo.\textsuperscript{12,13}

A second objective of this study was to estimate the annual detection rate of CL in Suriname in 2006. Vector-borne diseases like leishmaniasis, are thought to (re-) emerge in some areas due to environmental changes.\textsuperscript{15} In Suriname, deforestation and gold mining activities in the forested hinterland are increasing and there is a considerable high migration, mainly from gold miners from Brazil. CL is not an obligatory registered disease in Suriname, therefore no up-to-date data are available on prevalence or incidence of this disease. To meet the second objective, medical records were retrospectively reviewed at the dermatology clinics in Paramaribo, questionnaires were distributed to 55 medical posts in the forested hinterland and 7 of these medical posts were visited.

**Materials and methods**

**The study**

The study was reviewed and approved by the Medical Ethical Committee of the Academic Medical Center (AMC) in Amsterdam (MEC 03/228) and by the Ministry of Health in Paramaribo (VG 2006-001). One part of the study was conducted at the dermatology clinics (Dermatology Service of Ministry of Health and Dermatology Department, Academic Hospital) in Paramaribo, the capital, and the second part in the hinterland of the country in collaboration with the Medical Mission (MZ, Primary Health Care Suriname Paramaribo, Suriname).

**Paramaribo**

CL suspected patients were included prospectively at the dermatology clinics in Paramaribo from January to March and June to August 2006. Patients were included when informed consent was obtained and lesions were not confined to the face. They were interviewed, examined and 2-mm skin biopsies were collected from the indurated border of the lesion.
Patients were defined as CL positive when skin smear and/or PCR (hsp 70 or mini-exon) were positive. Additionally, a retrospective study was performed based on medical records to detect the number of CL patients (who were not included in the prospective study) who visited the dermatology clinics in Paramaribo in 2006.

**Hinterland**

Questionnaires were distributed to all 55 medical posts in the forested hinterland in 2006. The questionnaire included questions regarding age, gender, profession, ethnic group, medical history, (estimated) date of infection, start of symptoms, number and location of lesions and presence of lymphangitis. Health workers were asked to fill in a questionnaire for every new patient clinically diagnosed with CL, and to return these forms to the head office in Paramaribo.

To validate this method, seven medical posts in the forested hinterland in different parts of Suriname were visited from February to April 2006. Questionnaires were reviewed and current CL suspected patients were physically examined. Biopsies were collected from the indurated border of the CL suspected lesion.

**Parasite characterization**

Skin biopsies were stored in L6 lysis buffer (50 mM Tris HCl, 5 M GuSCN, 20 mM EDTA, 0,1% Triton-X-100) at -70°C at the Central Laboratory (Paramaribo, Suriname). After transport to KIT Biomedical Research (Amsterdam, The Netherlands), the samples were processed as described by Van der Meide et al.16 Nucleic acid extractions of the patient samples were analyzed in two PCR- Restriction Fragment Length Polymorphism (RFLP) methods; one assay was based on the spliced leader RNA gene (mini-exon) and the other on Heat Shock Protein 70 (hsp70) gene, as described by Marfurt et al.13 and Garcia et al.12

In each PCR run, the following reference strains were included as positive controls: Leishmania (Viannia) guyanensis MHOM/BR/75/M4147, L. (V.) braziliensis MHOM/BZ/75/M2903, L. (Leishmania) mexicana MHOM/MX/85/Solis, L. (L.) amazonensis MHOM/BR/81/LTB16, L. (V.) lainsoni MHOM/BR/86/M6426 and L. (V.) naiffi MHOM/00/94/CRE58.
Calculations

The proportion (upper and lower limit) of PCR positive patients in the study population in the forested hinterland was calculated by a 95% confidence interval (1.96 × SE). To estimate the detection rate of CL patients in Suriname the estimated number of CL patients (lower and upper limit) were divided by the number of inhabitants for the whole country (487,024) and forested hinterland (59,034) (numbers provided by the Medical Mission and General Bureau of Statistics, Paramaribo).

Results

Paramaribo

Thirty four CL suspected patients were included in the prospective study in Paramaribo. Thirty-three patients were defined as CL patients (based on microscopy and/or PCR), while one patient was tested negative, and excluded. Twenty-seven out of the 33 CL patients were positive in both mini-exon and hsp70 PCR; twenty-five patient samples were identified as *L. (V.) guyanensis*, one sample as *L. (V.) lainsoni* and one sample as *L. (L.) amazonensis*. Two patient samples were identified as *L. (V.) guyanensis* in mini-exon PCR-RFLP, but were negative in hsp70 PCR. Since *L. (V.) guyanensis* has the same fragment pattern as *L. (V.) lainsoni* in mini-exon PCR-RFLP, *L. (V.) lainsoni* can not be excluded as causative agent in these cases. Four patients were positive by microscopy, but negative with PCR. Geographical distribution and infecting *Leishmania* species of the 29 CL confirmed patients are presented in Table 1.

<table>
<thead>
<tr>
<th>Region of infection</th>
<th>(no, %)</th>
<th>Infecting species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper Suriname</td>
<td>1 (3%)</td>
<td><em>L. (V.) guyanensis</em></td>
</tr>
<tr>
<td>Brokopondo</td>
<td>17 (59%)</td>
<td><em>L. (V.) guyanensis (complex)</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>L. (V.) lainsoni</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>L. (L.) amazonensis</em></td>
</tr>
<tr>
<td>East Suriname</td>
<td>5 (17%)</td>
<td><em>L. (V.) guyanensis</em></td>
</tr>
<tr>
<td>West Suriname</td>
<td>2 (7%)</td>
<td><em>L. (V.) guyanensis</em></td>
</tr>
<tr>
<td>French Guiana</td>
<td>2 (7%)</td>
<td><em>L. (V.) guyanensis (complex)</em></td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (7%)</td>
<td><em>L. (V.) guyanensis</em></td>
</tr>
</tbody>
</table>