

## Case Report: First Case of Cutaneous Leishmaniasis Caused by *Leishmania (Viannia) braziliensis* in Suriname

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**Abstract.** The main causative agent of cutaneous leishmaniasis (CL) in Suriname is *Leishmania (Viannia) guyanensis*. This case report presents a patient infected with *Leishmania (Viannia) braziliensis*, a species never reported before in Suriname. This finding has clinical implications, because *L. braziliensis* has a distinct clinical phenotype characterized by mucocutaneous leishmaniasis, a more extensive and destructive form of CL that requires different treatment. Clinicians should be aware that chronic cutaneous ulcers in patients from the Guyana region could be caused by *L. braziliensis*.

### INTRODUCTION

The parasitic disease cutaneous leishmaniasis (CL) is an increasing health threat in Suriname and is caused by single-cell parasites of the genus *Leishmania*. In Suriname, *Leishmania* parasites cause a chronic infection with a spectrum of skin ulcers as the main clinical presentation. In Suriname, CL is known as boschyaw or boessie-yassi,<sup>1</sup> and the parasite *Leishmania (Viannia) guyanensis* is the most common cause of the disease<sup>2</sup>; the diversity of clinical CL forms suggests that other *Leishmania* species may be present in Suriname. Indeed, recent reports from Suriname confirm infection with *Leishmania (Viannia) lainsoni*, *Leishmania (Leishmania) amazonensis*, and *Leishmania (Viannia) naiffi*.<sup>3,4</sup> Variation in treatment outcome alongside parasite species diversity indicates that the standard therapy (pentamidine intramuscular [IM]) may not always be suitable. Therefore, identification of parasite species is important. Nucleic acid amplification tests (NAAT) are the standard techniques for species identification and have a high sensitivity and specificity.<sup>5,6</sup>

This report describes a patient who presented with ulcerative lesions after a hunting trip to West Suriname. With NAAT analysis, the patient proved to be infected with *Leishmania braziliensis*, a species of *Leishmania* not reported in Suriname until now.

### PATIENT, MATERIALS, AND METHODS

**Case report.** A 26-year-old male presented at the outpatient clinic of the Dermatology Service in Paramaribo (Suriname) in January 2010 with three nodular lesions with central ulceration, two (20 and 27 mm in diameter, respectively) on the right lower arm and one (12 mm in diameter) on the nostril (Figure 1A). He noticed the skin lesions 2 weeks after returning from a visit to the hinterlands in West Suriname in December 2009. Although he travels often to this area for fishing and hunting,

this is the first time he acquired these types of lesions. The lesions increased in size over the period of 1 month and the patient developed lymphadenopathy on the right lower arm. During the first visit, no apparent mucosal lesions were noticed.

Cutaneous leishmaniasis was suspected and lesional biopsy material was obtained. A Giemsa stain of biopsy smear, histopathology, and polymerase chain reaction (PCR) were all positive for *Leishmania* parasites. After written informed consent was obtained, the patient was entered into the PELESU study (Clinical, Parasitological, and Pharmaco-Economical evaluation of 3 days versus 7 days pentamidine isethionate regimen for cutaneous leishmaniasis in Suriname, Trial ID: NTR 2076). The patient received two IM injections of pentamidine each of 7 mg per kg body weight, with an interval of 2 days. After treatment the patient was followed up 6 and 12 weeks later to evaluate the healing process.

### CLINICAL SAMPLES

The lesion and the adjacent skin were cleaned and sterilized with disinfectant. Using a sterile disposable skin biopsy puncher, three skin biopsy samples of 2 mm in diameter were taken aseptically from the edge of the lesion according to the recommendation of the World Health Organization (WHO).<sup>7</sup> One biopsy was taken before treatment, one 6 weeks after treatment, and one 12 weeks after treatment, all from the same lesion. The biopsies were placed in 1 mL L6 lysis buffer consisting of 50 mM Tris HCl (Boehringer Ingelheim, Ridgefield, CT), 5 M guSCN (Fluka, Buchs, Switzerland), 20 mM EDTA (Tritriplex, Merck, Darmstadt, Germany), and 0.1% Triton-X-100 (Packard, Downers Grove, IL), and stored at –20°C.

*Leishmania* DNA extractions were performed according to the protocol described by Boom and others.<sup>8</sup> Briefly, the skin biopsy samples were disrupted by shaking with a 5-mm stainless steel bead in a mini-beadbeater-16 model 607 (Biospec Products, Bartlesville, OK) for 5 min. Next, the homogenates were collected and mixed for 5 min with 30 µL silica gel (SiO<sub>2</sub>, Sigma S5631; St. Louis, MO) to trap the DNA, and centrifuged for 15 sec at 12,000 × g. The silica pellet was collected, and washed repeatedly with L2 wash buffer (50 mM Tris HCl (Boehringer), 5 M guSCN (Fluka),

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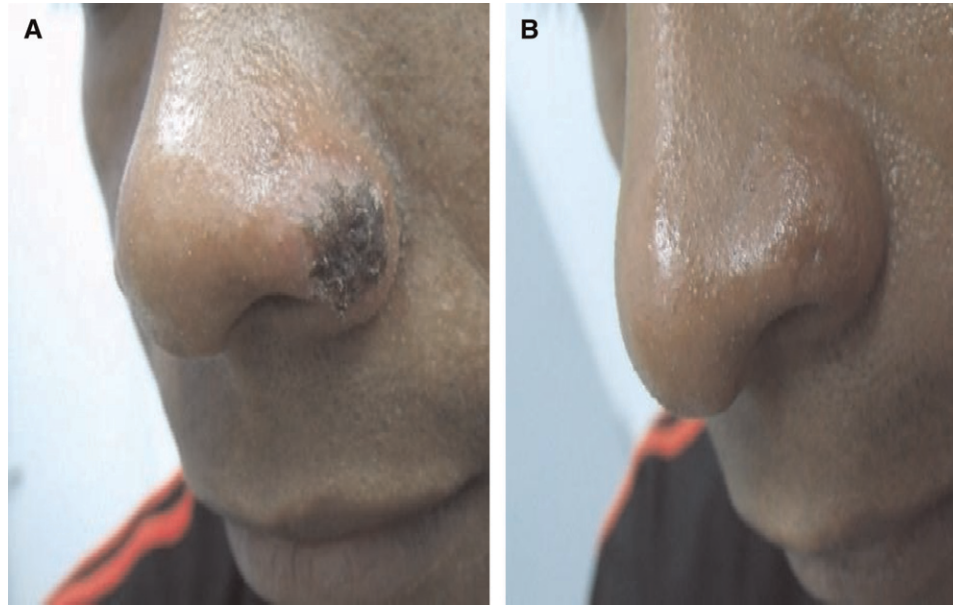


FIGURE 1. Ulcerative lesion with hemorrhagic crust caused by *Leishmania braziliensis* on the nostril of the patient, January–April 2010, Dermatologic Service, Paramaribo Suriname. (A) Before treatment and (B) 12 weeks after treatment with two doses of 7 mg/kg body weight pentamidine isethionate intramuscular (IM).

70% ethanol and acetone. The DNA was eluted in 100  $\mu$ L TE buffer (Tris EDTA buffer, 100 $\times$  concentrated Sigma) and stored at  $-20^{\circ}\text{C}$  until analysis.

To identify the infecting *Leishmania* species, a PCR-restriction fragment length polymorphism (PCR-RFLP) assay was performed on the spliced leader RNA gene PCR-RFLP (mini-exon) as described by Marfurt and others.<sup>9</sup> *Leishmania (Viannia) guyanensis* MHOM/BR/75/M4147 and *L. (Viannia) braziliensis* MHOM/BR/79/M2903 were included in the PCR run as reference DNA. After amplification, the PCR amplicons were digested using the restriction enzyme *HaeIII* (New England Biolabs, Ipswich, MA) for 2 h at  $37^{\circ}\text{C}$ . The resultant restriction digestions were analyzed on a 3% agarose gel and fragments were visualized by UV light.

To confirm that the patient was infected with *L. braziliensis*, sequencing of the mini-exon was performed using primers Fme2 and Rme2 (5'-ACT TTA TTG GTA TGC GAA ACT TCC GG-3' and 5'ACA GAA ACT GAT ACT TAT ATA GCG TTA G-3'). Products were sequenced using primer Rme2 (Macrogen Europe, Amsterdam, The Netherlands). The obtained nucleotide sequence was submitted to GenBank database under accession no. HE610677.

## RESULTS AND DISCUSSION

The PCR-RFLP and sequencing revealed that this patient was infected with *L. braziliensis*, a species previously not observed in Suriname. After pentamidine treatment, all lesions healed within 6 weeks without adverse events. At Week 12, all lesions had healed with atrophic scars and no relapse had occurred (Figure 1B). The patient did not return with relapse symptoms and could not be traced after completion of the study.

*Leishmania braziliensis* infection is often diagnosed in countries in South and Central America<sup>10,11</sup>; however, this is the first confirmed case in Suriname. In neighboring French

Guyana *L. guyanensis* is the main cause of CL, although cases caused by *L. braziliensis* are occasionally encountered.<sup>12</sup> Until recently it was believed that *L. guyanensis* was the only species prevailing in Suriname.<sup>13</sup> Because of the increasing travel between Suriname and neighboring countries, like Brazil and French Guyana, it is possible that other *Leishmania* species, such as *L. braziliensis*, have been imported.

The current treatment of CL in Suriname with pentamidine isethionate dates back to 1994 and has since then been used successfully.<sup>2</sup> Pentamidine isethionate (4 mg/kg body weight) intramuscularly is given three times with a 2- to 3-day interval. Recent observations of treatment failures and relapses after the pentamidine standard therapy (Lai A Fat RF, personal communication) indicates the emergence of reduced parasite susceptibility, or the possible introduction in Suriname of pentamidine-resistant species, other than *L. guyanensis*.

Here, the patient was successfully treated with pentamidine; however, this is not the preferred treatment of CL caused by *L. braziliensis* in Brazil and other Latin American countries. Pentavalent antimonials and recently oral miltefosine show fewer treatment failures and/or relapses.<sup>14,15</sup> For local treatment of *L. braziliensis* WHO recommends: 1) 15% paromomycin and 12% methylbenzethonium chloride ointment twice daily for 20 days, 2) thermotherapy: 1–3 sessions with localized heat ( $50^{\circ}\text{C}$  for 30 s), 3) intralesional antimonials: 1–5 mL per session every 3–7 days (1–5 infiltrations).<sup>16</sup> Recommended systemic treatment of *L. braziliensis* according to the WHO includes: 1) pentavalent antimonials; 20 mg  $\text{Sb}^{5+}$ /kg per day IM or intravenous for 20 days, 2) amphotericin B deoxycholate: 0.7 mg/kg per day, by infusion, for 25–30 doses, 3) liposomal amphotericin B: 2–3 mg/kg per day, by infusion, up to 20–40 mg/kg total dose.<sup>16</sup>

Although this patient showed no signs of mucosal involvement, it has been reported that 3–5% of patients with *L. braziliensis* develop mucocutaneous lesions (MCL) in Brazil.<sup>11,17</sup> In Suriname MCL caused by *L. guyanensis* is very

rare. Since we reported the occurrence of *L. braziliensis* in Suriname, clinicians need to be aware of possible mucocutaneous involvement in patients who contracted CL in Suriname.

In conclusion, *L. braziliensis* is present in Suriname. Here, a patient presented with ulcerations on the arms and nose, but no mucous lesions were observed. Treatment with pentamidine, the standard treatment of CL in Suriname, healed all lesions. Clinicians need to be aware of MCL caused by *L. braziliensis* in CL patients from Suriname. Further investigation and species identification is needed, especially for the patients who show treatment failure to the standard pentamidine regimen.

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