Cutaneous leishmaniasis: new developments in diagnosis and treatment evaluation
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Serodiagnosis of cutaneous leishmaniasis patients infected with \textit{Leishmania (Viannia) guyanensis} with homologous and heterologous antigens

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Submitted
**Leishmania (Viannia) guyanensis ELISA**

**Summary**

**Background.** An Enzyme-linked immunosorbent assay (ELISA) based on *Leishmania (Viannia) guyanensis* was developed to measure serum antibody levels to aid the diagnosis cutaneous leishmaniasis (CL) caused by *L. (V.) guyanensis*.

**Methods.** To detect antibody responses, the ELISA plate was coated with an antigen preparation of *L. (V.) guyanensis* promastigotes. Patients from Surinam (n=40) and Brazil (n=82) were included; all were infected with *L. (V.) guyanensis* as proven by PCR-Restriction Fragment Length Polymorphism. Also sera samples were tested from patients infected with *L. (Leishmania) chagasi* (n=62), *L. (L.) donovani* (n=30), *L. (L.) major* (n=27) and *L. (L.) tropica* (n=26). As negative controls sera samples were tested from healthy subjects (n=36), patients with other diseases (n=136) and patients with other kinetoplastid infections (n=27).

**Results.** The sensitivity of the ELISA was high; 95.0% for the Suriname patients and 81.7% for the Brazilian patients. No anti-*Leishmania*-specific antibody responses were found in 35 out of 36 healthy individuals and in 120 out of 136 human subjects with a variety of other diseases, confirming the high specificity of the assay. However, antibody responses were found in 15 out of 27 patients (55.5%) with other kinetoplastid infections. The assay performed equally well when using *L. (L.) donovani* promastigotes as capture antigen. However, with three other heterologous promastigote antigens (*L. (V.) braziliensis, L. (L.) amazonensis, * and *L. (L.) chagasi*) only 58% to 76% of the 122 *L. (V.) guyanensis* samples scored positive.

**Conclusions.** Our data are of great importance to improve the efficacy of serological diagnostic tests by the use of *L. (V.) guyanensis* or *L. (L.) donovani* antigen to monitor CL infections in the northeastern Amazon region where more than 90% of the CL patients are infected by *L. (V.) guyanensis*. 
Introduction

Leishmaniasis, caused by the intracellular protozoan *Leishmania* parasite, is a world wide disease with a prevalence of 12 million people, mainly in (sub-) tropical countries. The parasite multiplies in the macrophages of the host after the bite of an infected female sand fly of the genus *Phlebotomus* or *Lutzomya*. Clinical manifestations vary from ulcer or nodular skin lesions, to infection of mucosa or visceras and depend on the infecting species and the immunological status of the host. The cutaneous form, cutaneous leishmaniasis (CL), is most prevalent with 1-1.5 million new cases per year. CL is widespread in the northeastern Amazon region in South America, an area covering Guyana, Suriname, French Guiana en northern part of Brazil, and constitutes an important public health problem. In this region the disease is mainly a zoonosis, and human infection is primarily caused by *Leishmania (Viannia) guyanensis*. In a minority of cases infections are caused by *L. (V.) braziliensis* and *L. (Leishmania) amazonensis*, which can cause the mutilating mucocutaneous or disabling diffuse form, respectively.

A clear and disturbing increase in the number of CL cases is found in Brazil, but exact epidemiological data are lacking in Suriname and Guyana. One important factor contributing to this, is the establishment of new human settlements or extension of suburbs into the forest, which results in transmissions of infected sand flies from neighboring forests into human houses. Exposure to sand flies is further increased by deforestation, gold mining activities, building of dams or new irrigation schemes and migration. Therefore, health education and proper control strategies are important but lacking. For the control of CL, reliable diagnostic tests for the detection of the *Leishmania* parasites and suitable treatment are urgently needed.

Diagnosis of CL is often based on clinical picture and on visualization of the parasite in skin smears when a microscope is available. Alternative diagnostic methods are histopathology, culture and PCR. These assays are more laborious and well-equipped laboratory facilities are required. Serodiagnosis represents an easy, fast and cheap alternative and is more appropriate when handling large number of samples. Serological tests for diagnosis of visceral leishmaniasis (VL), including direct agglutination test (DAT)
and enzyme-linked immunosorbent assays (ELISAs) based on crude or recombinant antigens, are generally highly sensitive (>90%).8,9 Serodiagnosis for CL seems less suitable, presumably due to low antibody titers in blood.10 However, in recent years, the sensitivity of immunoassays have been improved showing that detectable antibody levels can be measured in CL patients.11,12

At present, there is still no agreement on the value of serologic tests for diagnosis in CL. ELISAs in current use are difficult to compare with each other, because great variability exists in antigen use and validation criteria. It can be argued that the use of a homologous antigen improves the performance of a serological assay, but there is a lack of studies comparing antigens of specific species with identified Leishmania infections. Serological assays for the New World are mainly based on L. (V.) braziliensis and L. (L.) amazonensis antigens11,13,14, while L. (V.) guyanensis causes >90% of CL infections in the northeastern Amazon region.4 For this reason, we examined the efficacy of a purified antigen preparation from L. (V.) guyanensis in an ELISA with human sera from patients with proven L. (V.) guyanensis infections. The antigen was tested with different sera panels, and compared with six other Leishmania antigens.

Materials and methods

Sera
The study population comprised cutaneous leishmaniasis (CL) suspected patients visiting Fundação de Medicina Tropical do Amazonas (FMTAM) in Manaus, Brazil and two Dermatology clinics (Dermatology Service, Ministry of Health and Dermatology Department of the Academic Hospital) in Paramaribo, Suriname. Sera samples of patients with CL were used in the study if the patients had lesions suspected of CL, no previous history of CL, provided written informed consent to participate in the study and positive Leishmania DNA isolation with identification of the causing species. Parasitological diagnosis was done by direct microscopic identification of Leishmania amastigotes in Giemsa stained skin smears, histopathology or PCR. Leishmania species identification was done on the basis of one of three PCR-RFLP assays (hsp70, mini-exon or ITS).15-17
Positive sera from patients infected with other *Leishmania* species were obtained from an established sera bank: 62 VL samples collected from VL patients in Brazil (*L. (L.) chagasi*), 30 from VL patients in Sudan (*L. (L.) donovani*), 27 *L. (L.) major* infected Dutch patients after a visit to Afghanistan and 26 *L. (L.) tropica* infected patients in Turkey.

Sera from healthy subjects from The Netherlands (n=36) with no history of CL or VL were tested as negative control group. For cross-reactivity study, 136 sera were tested from patients with other diseases; sarcoidose (n=17), tuberculose (n=20), schistosomiasis (n=11), toxoplasmosis (n=20), toxocariasis (n=17), amoebiasis (n=16), giardiasis (n=7) and malaria (n=28). A third control group consisted of 27 patients infected with related kinetoplastid protozoa; *Trypanosoma gambiensis* (n=16) and *Trypanosoma cruzi* (n=11).

All sera samples were collected before treatment and inactivated at 56 °C for 30 minutes and subsequently stored at –20 °C. Each sample had its unique sample code. The study was reviewed and approved by the Medical Ethical Committee of the Academic Medical Center (AMC) in Amsterdam (MEC 03/228) and the Brazilian national review board of the Ministry of Health (Commissão Nacional de Ética em Pesquisa, CONEP, Parecer Nº 1142/2005).

**Antigen preparation**

The following 7 antigens were used to perform the ELISA: *L. (V.) guyanensis* (MHOM/SR/2005/KIT27), *L. (V.) braziliensis* (MHOM/BR/86/WR675), *L. (L.) amazonensis* (MHOM/BR/81/LTB16), *L. (L.) major* (MHOM/IR/72/NADIM5), *L. (L.) tropica* Turkey (MHOM/TR/E18), *L. (L.) donovani* (MHOM/SD/68/1S) and *L. (L.) chagasi* (MCAN/BR/84/C0910).

All the above mentioned species, were maintained as promastigote cultures in RPMI 1640 medium (Gibco, Scotland), supplemented with 15% fetal calf serum and 5000 U/ml Penicillamine and 5000 μg/ml Streptomycin at the Royal Tropical Institute. The parasites were weekly subcultured.

From each culture, promastigotes were harvested from a 6-day old culture in 585 ml medium and washed in 100 ml phosphate-buffered saline (PBS; pH 7.2). After centrifugation (3,000 rpm for 15 minutes at room temperature), the supernatant was
removed and the pellet was reconstituted in 10 ml PBS. The suspension was vortexed thoroughly and sonicated on ice with macrotip (level 8, with a 50% interval, 15 pulses). Subsequently, 5 ml PBS was added and the suspensions were centrifuged (14,000 rpm for 30 minutes at room temperature). The supernatant was collected, aliquoted into clean 1.5-ml tubes and stored at –20ºC. Protein concentration was determined with the BCA™ Protein Assay Kit (Pierce). The optimum working concentration of antigens, sera and antihuman serum conjugates were determined with checkerboard titration method.

ELISA

The ELISAs were performed in 96-well polystyrene flat-bottom microtiter medium-binding plates (Greiner, Germany). The plates were coated with 100 μl antigen preparation per well (see Table 1) and incubated overnight at room temperature. The plates were washed three times with PBS / 0.1% Tween-20 (pH 7.2), followed by a blocking step of 45 minutes at 37ºC with 150 μl PBS supplemented with 3% bovine serum albumine (BSA). The plates were washed three times with the same buffer mentioned above and incubated with 100 μl serum dilution (1:400 or 1:800 dilution [Table 1] in PBS 2% casein / 1% BSA / 0.05% Tween-80) for 60 minutes at 37ºC.

Table 1. Overview Leishmania antigens and sera dilutions

<table>
<thead>
<tr>
<th>Coatings concentration (μg per well)</th>
<th>Sera dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. (V.) guyanensis</td>
<td>0.5</td>
</tr>
<tr>
<td>L. (V.) braziliensis</td>
<td>0.5</td>
</tr>
<tr>
<td>L. (L.) amazonensis</td>
<td>1</td>
</tr>
<tr>
<td>L. (L.) chagasi</td>
<td>0.5</td>
</tr>
<tr>
<td>L. (L.) donovani</td>
<td>1</td>
</tr>
<tr>
<td>L. (L.) major</td>
<td>0.5</td>
</tr>
<tr>
<td>L. (L.) tropica</td>
<td>2</td>
</tr>
</tbody>
</table>

After three washings, 100 μl Horse Radish Peroxidase-conjugated goat polyclonal antihuman IgG (Jackson) dilution (1:40.000 in PBS / 1% BSA / 0.1% Tween-20) was
added for 60 minutes at 37°C. After another cycle of three washings, 100 μl of TMB (3,3′,5,5′-tetramethylbenzidine) substrate was added followed by 100 μl 0.5 N H₂SO₄ to stop the reaction after 20 minutes incubation at room temperature. The optical density (OD) was measured in an ELISA reader (Sunrise TECAN) at 450 nm. The assays were performed over 7 days.

**Statistical analysis**

The cut-off point (lower limit of detection) for each antigen was established as the average absorbance at 450 nm plus 3 standard deviations (SDs) of the 36 sera from healthy subjects with no history of CL or VL. One positive sera were used in all plates as reference. The results were calculated as arbitrary ELISA units to account for day-to-day variation; \[ \text{OD}_{\text{sample}} \times (\text{OD}_{\text{day1}}_{\text{reference sera}} / \text{OD}_{\text{day y}}_{\text{reference sera}}) \]. The sensitivity and specificity of the assay was calculated for each antigen and sera panel separately. The sensitivity was calculated with the formula: \( TP / (TP + FN) \); the specificity was calculated with the formula \( TN / (TN + FP) \) (TP = True Positive, FN = False Negative, TN = True Negative and FP = False Positive). Statistical Package for Social Sciences (SPSS version 14.01) was used to compare not normal distributed data by the Mann-Whitney U-Test, and the Chi-square test for proportions. Test results obtained with different antigens were compared using Kappa agreement test.

**Results**

In total, 82 patients from Brazil and 40 patients from Suriname with proven *L. (V.) guyanensis* infection were included in the study. Table 2 shows patient characteristics of the two study groups. A significant difference was only observed regarding the duration of disease (p<0.05), with the Suriname group in general 3 weeks longer infected than the Brazilian group. Mean OD values were significantly higher in patients with disease of more than 4 weeks duration and in the presence of nodular lymphangitis (p<0.05).
**Leishmania (Viannia) guyanensis ELISA**

**Table 2.** Comparison of the main characteristics of CL patients infected with *L. (V.) guyanensis* from Suriname and Brazil.

<table>
<thead>
<tr>
<th></th>
<th>Suriname patients (n=40)</th>
<th>Brazil patients (n=82)</th>
<th>Statistic (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (years, range)</td>
<td>30.0 years (14-58)</td>
<td>28.5 years (14-80)</td>
<td>0.935</td>
</tr>
<tr>
<td>No. of male (%)</td>
<td>37 (92.5%)</td>
<td>66 (80.5%)</td>
<td>0.115</td>
</tr>
<tr>
<td>Median no. of lesions (range)</td>
<td>2 (1-16)</td>
<td>2 (1-16)</td>
<td>0.441</td>
</tr>
<tr>
<td>Median duration of disease (weeks, range)</td>
<td>7 (2-44)</td>
<td>4 (2-52)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>No. of patients with nodular lymphangitis (%)</td>
<td>26/38* (68.4%)</td>
<td>45/80* (56.3%)</td>
<td>0.207</td>
</tr>
</tbody>
</table>

Values are number of patients or medians with ranges in parenthesis (Mann-Whitney U for medians and Chi-square statistic for proportions)

* Presence of nodular lymphangitis in two patients was unknown

**Table 3.** Sensitivity and specificity of anti-*L. (V.) guyanensis* IgG antibody ELISA in the diagnosis of different patient groups infected with variable *Leishmania* species.

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Infecting <em>Leishmania</em> Species</th>
<th>Antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suriname CL patients (n=40)</td>
<td><em>L. (V.) guyanensis</em></td>
<td>38/40 (95.0%)</td>
</tr>
<tr>
<td>Brazil CL patients (n=82)</td>
<td><em>L. (V.) guyanensis</em></td>
<td>67/82 (81.7%)</td>
</tr>
<tr>
<td>Dutch CL patients Afghanistan (n=27)</td>
<td><em>L. (L.) major</em></td>
<td>8/27 (29.6%)</td>
</tr>
<tr>
<td>Turkish CL patients (n=26)</td>
<td><em>L. (L.) tropica</em></td>
<td>10/26 (38.5%)</td>
</tr>
<tr>
<td>Sudan VL patients (n=30)</td>
<td><em>L. (L.) donovani</em></td>
<td>27/30 (90.0%)</td>
</tr>
<tr>
<td>Brazilian VL patients (n=62)</td>
<td><em>L. (L.) chagasi</em></td>
<td>54/62 (87.1%)</td>
</tr>
</tbody>
</table>

**Sensitivity**

<table>
<thead>
<tr>
<th>Positive/total (%)</th>
<th>35/36 (97.2%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-endemic healthy controls (n=36)</td>
<td>-</td>
</tr>
<tr>
<td>Other diseases (n=136)</td>
<td>120/136 (88.2%)</td>
</tr>
<tr>
<td>Related Kinetoplastids (n=27)</td>
<td>12/27 (44.4%)</td>
</tr>
</tbody>
</table>

**Specificity**

Sensitivities and specificities of the different patient groups tested in ELISA with *L. (V.) guyanensis* antigen are presented in Table 3. Significant higher levels (p<0.001) of anti-Leishmanial IgG antibodies were detected in the Suriname patient group in comparison with the Brazilian group. In total 38 out of 40 CL patients were seropositive with a mean absorbance (OD) of 0.982 ± 0.426. From the Brazil CL patient group 67 out of 82 patients were above cut-off value with a mean OD of 0.651 ± 0.346. When patients infected with other *Leishmania* species were tested with *L. (V.) guyanensis* antigen, lower absorbance
values (and thus sensitivities) were found for the patients infected with *L. major* with a mean OD of 0.258 ± 0.110 (sensitivity of 29.6%) or *L. (L.) tropica* with a mean OD of 0.337 ± 0.195 (sensitivity of 38.5%). Higher absorbance values were observed for the VL patients from Sudan (*L. donovani*) with a mean OD of 0.689 ± 0.319 (sensitivity of 90.0%) and for VL patients (*L. chagasi*) from Brazil a mean OD value of 0.342 ± 0.737 (sensitivity of 87.1%).

In 36 healthy individuals from non-endemic country, tested with *L. (V.) guyanensis* antigen, specificity was very high (97.2%), with a low mean OD of 0.153 ±0.061. OD values were higher for the patient group (n=136) with other diseases (mean OD of 0.196 ± 0.109). The OD values were high (mean OD of 0.431 ±0.333) for the patients with other kinetoplastid infections (Chagas disease and African trypanosomiasis).

<table>
<thead>
<tr>
<th>Antigen Leishmania species</th>
<th>Suriname and Brazilian patients (n=122)</th>
<th>Non-endemic healthy individuals (n=36)</th>
<th>Other diseases (n=136)</th>
<th>Related kinetoplastids (n=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. (V.) guyanensis</em></td>
<td>105/122 (86.0%)</td>
<td>35/36 (97.2%)</td>
<td>120/136 (88.2%)</td>
<td>12/27 (44.4%)</td>
</tr>
<tr>
<td><em>L. (V.) braziliensis</em></td>
<td>93/122 (76.2%)</td>
<td>35/36 (97.2%)</td>
<td>124/136 (91.2%)</td>
<td>13/27 (48.1%)</td>
</tr>
<tr>
<td><em>L. (L.) amazonensis</em></td>
<td>71/122 (58.2%)</td>
<td>35/36 (97.2%)</td>
<td>127/136 (93.4%)</td>
<td>17/27 (63.0%)</td>
</tr>
<tr>
<td><em>L. (L.) major</em></td>
<td>101/122 (82.8%)</td>
<td>34/36 (94.4%)</td>
<td>117/136 (86.0%)</td>
<td>14/27 (51.9%)</td>
</tr>
<tr>
<td><em>L. (L.) tropica</em></td>
<td>98/122 (80.3%)</td>
<td>35/36 (97.2%)</td>
<td>122/136 (89.7%)</td>
<td>14/27 (51.9%)</td>
</tr>
<tr>
<td><em>L. (L.) donovani</em></td>
<td>106/122 (86.9%)</td>
<td>35/36 (97.2%)</td>
<td>121/136 (89.0%)</td>
<td>11/27 (40.7%)</td>
</tr>
<tr>
<td><em>L. (L.) chagasi</em></td>
<td>84/122 (68.9%)</td>
<td>35/36 (97.2%)</td>
<td>124/136 (91.2%)</td>
<td>15/27 (55.6%)</td>
</tr>
</tbody>
</table>

The *L. (V.) guyanensis* antigen was compared with 6 other non-homologue antigens. Sensitivities and specificities are presented in Table 4. Cut-off values, based on the healthy control group (n=36), was calculated for each antigen separately (specificity ranging between 94.4% and 97.2%). The specificity for the patient group with other diseases (n=136) ranged between 86.0% and 93.4%. While specificity was highest for *L. (L.) amazonensis* antigen, sensitivity was only 58.2%. To the contrary, *L. (L.) donovani* had the
highest sensitivity (86.9%) and a specificity of 89.0% (in comparison with *L. (V.) guyanensis*; sensitivity of 86.0% and specificity of 88.2%). Specificity for the third control group with kinetoplastid related infections was low, ranging between 40.7% and 63.0%. Overall, highest agreement (94.2%) was found between *L. (V.) guyanensis* and *L. (L.) tropica* antigen (kappa=0.796, p<0.001), with 98 patients (80.3%) showing positive and 17 patients (13.9%) negative concordant results in ELISA. Lowest agreement (70.5%) was found with *L. (L.) amazonensis* antigen (kappa=0.331, p<0.001), with only 70 patients (57.4%) showing positive and 16 patients (13.1%) negative concordant results.

**Discussion**

In the northeastern Amazon region, most cutaneous leishmaniasis (CL) patients are infected with *Leishmania (Viannia) guyanensis*, but serological assays are often based on *L. (Leishmania) amazonensis* and *L. (V.) braziliensis* antigens. This study shows that patients infected with *L. (V.) guyanensis* responds much better to *L. (V.) guyanensis* and *L. (L.) donovani* antigens (sensitivities of 86.0 % and 86.9%, respectively), than to *L. (L.) amazonensis*, *L. (V.) braziliensis* and *L. (L.) chagasi* (sensitivity of 58.2%, 68.9% and 76.2%, respectively) antigens.

Comparison of *L. (V.) guyanensis* antigen with two different *L. (V.) guyanensis* sera panels demonstrated that the antigen yielded higher mean absorbance values in the Suriname patient group than in the patient group from Brazil. The higher level of IgG antibodies in the Suriname group could be due to the longer duration of CL disease. The median disease duration in the Suriname group was 7 weeks in comparison with 4 weeks for the Brazilian group. When we compared patients with short (less than 4 weeks) and long (more than 4 weeks) duration of disease we did found a significant higher antibody level in patients with a long duration. However, the antigen *L. (V.) guyanensis* used in this study was isolated from a Suriname patient in 2005 and the better performance in the Suriname group could therefore be related to the greater homology. Better performances by using homologous antigen has been reported previously, although also contradictory results have been found. One ELISA for diagnosis of American visceral leishmaniasis
used antigen prepared from two different *L. (L.) chagasi* isolates from different regions.\(^9\)

Comparison among the *L. (L.) chagasi* isolates demonstrated that the antigen prepared with the isolate from the same area where the sera originated yielded higher mean absorbance than the other. Intra-species variation could play a role and has been described within the *L. (V.) guyanensis* species, what could influence the serodiagnosis of *L. (V.) guyanensis* infected patients from different regions.\(^{20,21}\)

Our results showed that *L. (V.) guyanensis* and *L. (L.) donovani* antigens performed equally well in *L. (V.) guyanensis* infected patients. On the other hand, *L. (L.) amazonensis*, *L. (V.) braziliensis* and *L. (L.) chagasi* antigens showed relatively low cross-reactivity with antibodies from *L. (V.) guyanensis* infected patients. Since all antigens were prepared under identical technical conditions, variations in antigenic quality may depend on factors inherent to the particular species of the parasite. Serological diagnosis of CL in Brazil is often based on *L. (L.) amazonensis* and *L. (V.) braziliensis* antigens\(^{11,13,14}\), particularly the former since this species is easier to cultivate than *Viannia* species. A few studies have demonstrated that antibody responses in *L. (V.) braziliensis* infected patients are higher than those raised in *L. (V.) guyanensis* or *L. (V.) panamensis* infected patients based on *L. (L.) amazonensis* or *L. (V.) braziliensis* antigen.\(^{11,22}\) How high antibody responses against *L. (V.) guyanensis* antigen would have been in these studies, is not known. Based on our results, the outcome could have been very different in terms of CL positivity. This confirms the urgent need of testing serodiagnostic assays based on *L. (V.) guyanensis* antigen, in areas where this species causes most CL infections, as in the Northeastern Amazon region.

Specificity of the assay was high when healthy controls from non-endemic country were used. Additionally, a large panel of 136 sera samples was tested from patients with other diseases, of which only a few patients were positive (11.8\%). For positive tested patients in our study previous exposition to *Leishmania* parasites cannot be ruled out. The specificity of our assay is low when sera from patients infected with *Trypanosoma* are tested. It is well known that specific proteins of *Leishmania* are recognized by serum of Chagas disease patients.\(^{23,24}\) Attempts should be made to increase specificity of the assay. This may be done by using another IgG isotype in stead of total IgG, as demonstrated by Pedras et al..\(^{14}\)
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However, sensitivity may be affected. In general, previous decision analysis has shown that a serological test with a high sensitivity and “acceptable” specificity is a better alternative for case detection algorithms than are tests with insufficient sensitivity, such as skin smears.25

In conclusion, the purpose of the study was to compare different antigens with \textit{L. (V.) guyanensis} in proven \textit{L. (V.) guyanensis} infected patients. While many serological assays are still based on \textit{L. (L.) amazonensis} and \textit{L. (V.) braziliensis} antigens, we showed that the use of \textit{L. (V.) guyanensis} improved the assay significantly in \textit{L. (V.) guyanensis} infected patients. Our study showed that antibody detection with \textit{L. (V.) guyanensis} and \textit{L. (L.) donovani} antigen constitutes a valuable alternative assay to increase the efficiency of serological diagnosis of CL patients in the northeastern Amazon region, what can also be useful in epidemiological surveys.

Acknowledgments

We gratefully thank the patients for their participation, time and patience in this study. We thank the staff of the Leishmaniose Laboratory at FMTAM in Manaus and of the dermatology clinics in Paramaribo who helped with translations and registration of patients. Further, we thank Patricia van Sambeek, Esther Pariama, Alexa ter Horst and Jetske Emmelkamp (AMC, Amsterdam, The Netherlands) for collection of data and patient samples. We thank Tom van Gool (Dep. of Parasitology, AMC, The Netherlands), Yusuf Özbek (Dep. of Parasitology, Ege University, Izmir, Turkey) and Eduardo S. Da Silva (Universidade Federal de São João, São João Del Rei, Brazil) for storing their sera samples in the sera bank. This work was supported by a grant from The Netherlands Foundation for the Advancement of Tropical research (WOTRO contract 96-210).
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