Tropical medicine rounds

Clinical features of cutaneous and disseminated cutaneous leishmaniasis caused by *Leishmania* (*Viannia*) *braziliensis* in Paraty, Rio de Janeiro

Ricardo Vieira-Gonçalves, MD, Claude Pirmez, MD, PhD, Maria Eugenia Jorge, MD, Wilson Jacinto Silva Souza, MD, PhD, Márcia Pereira Oliveira, BcS, PhD, Marcio S. Rutowitsch, MD, PhD, and Alda Maria Da-Cruz, MD, PhD

From the Laboratório de Imunoparasitologia and Laboratório de Imunopatologia, Instituto Oswaldo Cruz/FIOCRUZ, Centro Integrado de Saúde, Secretaria Municipal de Saúde de Paraty, and Hospital dos Servidores do Estado, Ministério da Saúde, Rio de Janeiro, Brazil

Correspondence
Alda Maria Da-Cruz, MD, PhD
Laboratório de Imunoparasitologia
Instituto Oswaldo Cruz, Av. Brasil 4365
Pav. Leônidas Deane 4° andar Manguinhos
CEP 21040-900
Rio de Janeiro
Brazil
E-mail: alda@ioc.fiocruz.br

Abstract

Background American tegumentary leishmaniasis (ATL) caused by *Leishmania (Viannia) braziliensis* is endemic in Rio de Janeiro State (RJ), where the disease shows epidemiologic and clinical characteristics distinct from those of ATL in other Brazilian regions. Paraty is the second most important endemic area in RJ; however, reports on leishmaniasis in this region refer to the occurrence of the disease without describing its characteristics.

Methods The clinical features of 71 cases of ATL reported between 1991 and 1997 in Paraty are presented. Thirty patients were re-evaluated 10 years later.

Results Males and females were affected in similar proportions, and the disease was more prevalent in patients aged between 10 and 49 years (63.4%). Cutaneous leishmaniasis was the most prevalent clinical form observed. Unique lesions were present in 69% of cases, 91.6% of which displayed an ulcerated aspect. Although mucosal leishmaniasis was not observed, severe clinical manifestations, such as disseminated cutaneous lesions caused by *L. braziliensis*, were diagnosed in two patients. These patients presented skin lesions with different clinical aspects spread throughout the body, as well as low cellular immune responses. Montenegro skin test (92% positivity) and serology (8% IgM and 56% IgG anti-*Leishmania* positive results) were the most utilized tests for supporting the diagnosis of leishmaniasis. Parasites, detected in 27 of the 33 cases analyzed, were characterized as *L. braziliensis*.

Conclusion ATL in Paraty shares the clinical and laboratory characteristics reported for ATL in other regions of RJ, probably because of the similar epidemiologic context related to the Atlantic rainforest region.

Introduction

American tegumentary leishmaniasis (ATL) is a vector-borne infectious disease caused by intracellular protozoan parasites of the genus *Leishmania*. According to the World Health Organization (WHO), leishmaniasis is endemic in 88 countries, and 350 million people are estimated to be at risk of contracting this disease. Brazil presents the highest prevalence of leishmaniasis in the Americas, where around 30,000 cases are recorded each year,[1] therefore representing a serious public health problem.

The clinical forms of ATL include cutaneous leishmaniasis (CL), mucosal leishmaniasis (ML), and diffuse cutaneous leishmaniasis (DCL). Moreover, unusual manifestations have been described, such as leishmaniasis recidiva cutis and disseminated cutaneous leishmaniasis (DissL).[2–4] *Leishmania* (*Viannia*) *braziliensis* is the most prevalent species in all geographic regions in Brazil; nevertheless, other species, such as *Leishmania* (*Leishmania*) *amazonensis* and *Leishmania* (*Viannia*) *guyanensis*, are also frequently isolated from human cases.[5,6]

Leishmaniasis is endemic in Rio de Janeiro State (RJ), and the disease has been present in the region since the end of the 19th century.[7] The endemic region has expanded out of the original focus in the rainforest and rural areas, reaching peri-urban zones.[8] This expansion of the endemic region explains the increase in the number of recorded cases. Nowadays, the infection exhibits both peri-urban and rural routes of transmissiom;[9] moreover, peri-domicile transmission plays a more important role than the classical sylvatic

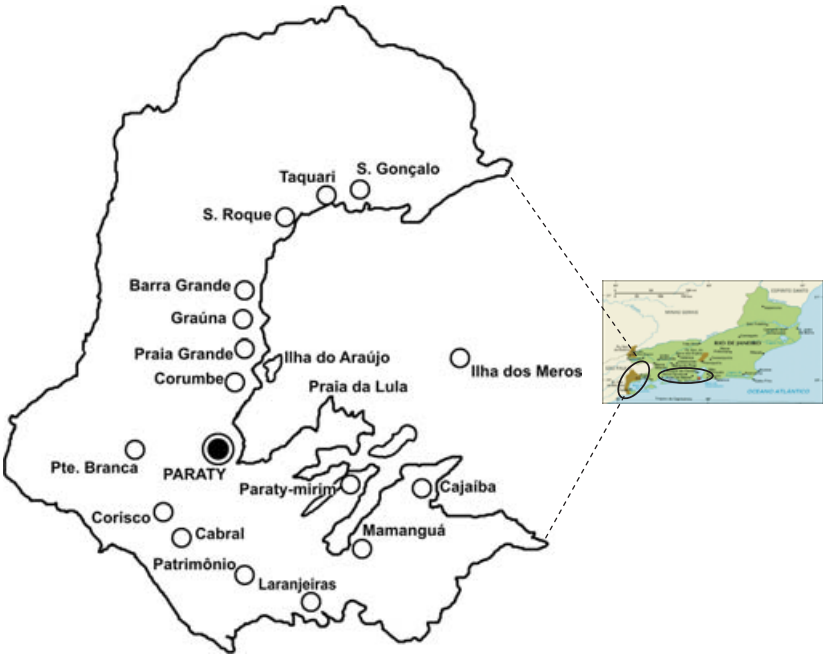

Figure 1 Map of Paraty municipality with the principal localities of the patients. The small map shows Rio de Janeiro State and the circles highlight the main endemic areas for leishmaniasis – Rio de Janeiro and Paraty cities

transmission cycle. The most prevalent clinical manifestation is CL and the mucosal disease is rarely found.[9,10] *L.* (*V.*) *braziliensis* is the most prevalent species encountered in the region.[9,11]

The cities of Rio de Janeiro and Paraty represent the two major endemic areas of ATL in RJ, both located within the Atlantic rainforest region (Fig. 1).[1] The first cases of leishmaniasis in Paraty (five patients) were identified during an epidemiologic survey carried out amongst 1612 individuals from different endemic areas of RJ.[12] Infected dogs were identified in the region, corroborating the importance of the peri-domicile cycle in transmission.[13] The sandfly species responsible for parasite transmission include *Lutzomyia intermedia*, *Lu. migonei*, and *Lu. fischeri*.[14]

The first cases of leishmaniasis officially reported in Paraty date from 1988, even though compulsory notification started in 1980 (Fig. 2).[1] There was a rapid increase in case reports after 1989, with a registered peak in 1991 (134 cases). The disease became endemic, and a decreasing trend was apparent after 1997. The main period of disease transmission occurred between 1990 and 1996 when 504 cases of leishmaniasis were recorded (Fig. 2).

The epidemiologic importance of leishmaniasis in RJ emphasizes the need for new studies addressing the clinical features of ATL, in particular in the endemic region of Paraty. The prevention of, and therapeutic and control programs for, leishmaniasis depend on a knowledge of the disease. The available reports on human leishmaniasis in this region, however, refer to the occurrence of the disease without describing its characteristics,[12] or involve isolated case reports.[15]

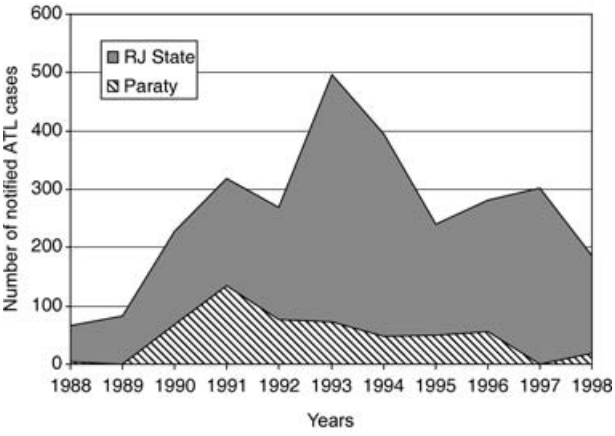

Figure 2 Annual incidence (1988–98) of American tegumentary leishmaniasis (ATL) in Paraty in relation to that notified in Rio de Janeiro (RJ) State, as reported to the Brazilian National Health Secretary, Ministry of Health

Therefore, our aim was to identify the main clinical characteristics of leishmaniasis in Paraty through a retrospective study of 71 confirmed cases of ATL.

Methodology

Study area

Paraty is a historical city located 236 km south of Rio de Janeiro, 5 m above sea-level, with a total area of 928 km^2. It is located

within the Atlantic rainforest region, with a tropical climate. The main economic source is tourism. Our study group represents a sample of the 30,000 total inhabitants living in the region at the time, mostly located in rural or peri-urban areas.

Patients

One hundred and thirty-two patients from rural or peri-urban areas of Paraty (Fig. 1), who attended the Posto Municipal de Saúde Dr Derly Ellen (Centro Integrado de Saúde de Paraty) between January 1991 and December 1997, were selected for this study. Seventy-one patients fulfilled the inclusion criteria of a clinical and epidemiologic history compatible with ATL, plus at least one positive laboratory examination for the diagnosis of leishmaniasis.[16] Patients were submitted to a complete clinical examination including epidemiological history, focusing on the age, sex, and residence, and physical and dermatologic examination. Clinical aspects, such as the duration of illness before diagnosis, lesion characteristics (area, number of affected sites, anatomic region, and shape of lesion), and lymphatic involvement, were also evaluated. Thirty patients were re-evaluated in 2006 for clinical evolution (cure or reactivation of the disease). This study was conducted in agreement with the international guidelines for biomedical research, and was approved by the Ethical Committee of the Fundação Oswaldo Cruz, Ministério da Saúde, Brazil.

Diagnostic procedures

The following laboratory criteria were adopted for the diagnosis.

1 Delayed-type hypersensitivity (DTH) to leishmanial antigens (Montenegro skin test, MST). A volume of 0.1 mL of *Leishmania* promastigote antigens (provided by the Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais) was injected intradermally into the internal side of the left forearm. After 48 h, the presence of an induration reaction with a major width of 5 mm or more was considered to be positive. DTH was also evaluated by *in vitro* assays of the lymphocyte proliferative response (LPR) to leishmanial antigens.[17]

2 *Leishmania*-specific antibodies were detected by indirect immunofluorescence assays. Reciprocal antibody titers ≥ 45 were considered to be positive.[18]

3 Detection of *Leishmania* parasites and histopathology. Biopsy was performed at the active border of the lesion. The sample was divided into fragments, which were submitted to at least one of the following procedures: (i) Leishman-stained imprints of tissues for direct detection of the parasite; (ii) histopathologic analysis (hematoxylin and eosin) of the inflammatory infiltrate; and/or (iii) isolation of *Leishmania* parasites by culture in Nicolle–Nevy–McNeal (NNN) modified medium. For *in vitro* culture, the fragments were maintained in phosphate-buffered saline (PBS) plus antimicrobials (penicillin and streptomycin) for at least 4 h before being processed. The species of isolated parasite were characterized by isoenzyme electrophoresis profiles.[11] To confirm the diagnosis, detection of DNA by polymerase chain reaction (PCR) and hybridization with probes from a cloned minicircle of *L.* (*V.*) *panamensis* or *L.* (*L.*) *amazonensis* were performed.[19]

Results

The 71 patients included in this study had a confirmed diagnosis of ATL.[16] The disease was equally distributed between males (47.9%) and females (52.1%). The mean age ± standard deviation (SD) was 22.8 ± 13.1 years (median, 21.3 years; range, 6 months to 73 years). Patients aged between 10 and 49 years accounted for 63.4% of the reported cases; 15 patients were less than 9 years of age and 10 patients were more than 50 years of age. The mean duration of the disease was 79.8 ± 78.8 days (median, 60 days; range, 7 days to 1 year). The duration of disease was distributed as follows: 36%, up to 30 days; 28%, 31–60 days; 14%, 61–90 days; 8%, 91–120 days; 14%, 120 days to 1 year. The majority of patients (78%) sought clinical care within 90 days of lesion evolution.

CL was the most prevalent clinical form reported (69 cases), the majority (49 cases) with a single lesion; 20 patients presented two to ten lesions. The lesions were predominantly observed on the upper (37%) or lower (36%) limbs. The face was affected in 23% of cases, especially in the first decade of life (61.5%). The mean lesion size was 5.2 ± 5.6 cm^2, ranging from 0.2 to 24.7 cm^2. Ulceration was the most frequent clinical aspect observed in the patients (91.6%).

Two patients exhibited DissL lesions. One had a papular lesion located on the upper extremity which progressed to an ulcer after local trauma (Fig. 3d). The ulcer, measuring 3.0 × 2.5 cm, was not painful and had a granular aspect containing a whitish exudate. Lymphadenopathy was present (Fig. 3d). After 2 weeks, papulomatous pruriginous lesions spread throughout the body, including the face, but without mucosal involvement (Fig. 3a–c). The lesions, ranging in size, were predominantly ulcerated and verrucous. Despite antimonial therapy, the disseminated lesions progressed and worsened (Fig. 3e,f). The other patient presented with two ulcerated lesions on the right foot that evolved to erythematous papules scattered over the face, body, and limbs, each measuring approximately 0.5 cm in diameter. Edema and erythema of the nose with blood fluid drainage were also observed. Both patients had negative serologic tests for human immunodeficiency virus (HIV) infection.

With regard to the laboratory findings, MST was positive in 58 of the 63 patients tested (mean size, 15.7 ± 10.9 mm). Anti-*Leishmania* antibodies were detected in 56% of patients: IgG, 34 cases (48%; titers ranging from 1 : 45 to 1 : 180); IgM plus IgG, six cases (8%; titers ranging from 1 : 45 to 1 : 90). Both DissL patients had positive MST and IgG antibody titers (1 : 45). The *in vitro* LPR to *Leishmania* antigen stimulation was positive in all 18 cases of CL studied (mean stimulation index, 27 ± 26; median, 13.3; range, 3–81.3), but the two DissL patients showed a negative LPR.

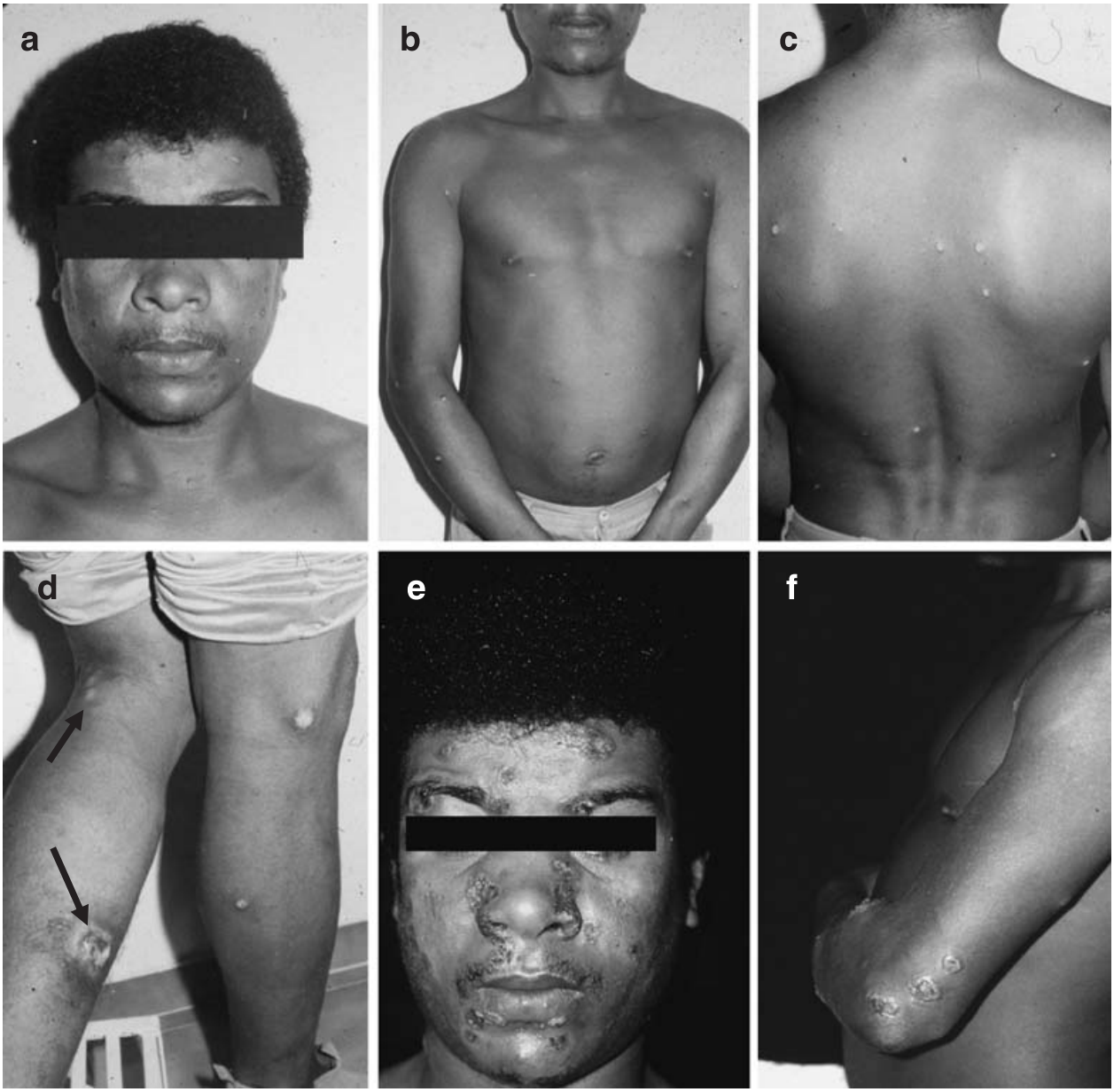

Figure 3 Disseminated cutaneous leishmaniasis caused by *Leishmania* (*Viannia*) *braziliensis*. Lesions were scattered over the face (a), trunk (b, c), and arms and legs (b, d). The ulcerated lesions progressively worsened despite antimonial therapy (e, f). The arrows indicate the initial ulcerated lesion and lymphadenopathy (d)

The parasite was isolated by culture in 78.5% of the 28 skin lesions analyzed. The detection of *Leishmania* DNA by PCR was positive in 16 of the 18 skin samples studied. Identification by zymodeme analysis was possible for only eight cases, all characterized as *L.* (*V.*) *braziliensis*.

Histopathologic analysis of the CL lesions (20 cases) displayed predominantly a lymphoplasmocytic infiltrate. Early granuloma formation was detected in eight cases, and typical tuberculoid granulomas were observed in four lesions. Amastigotes were visualized in only two samples. DissL did not present significant histopathologic differences from CL.

Thirty patients were re-evaluated around 10 years after the end of therapy. Twenty-five cases maintained healed lesions. Five patients returned to the clinical unit because of a new episode of leishmaniasis during this period.

Discussion

Clinical variations of ATL are observed between different endemic areas affected by the same parasite species.[8,9,20–26] Thus, studies of the clinical characteristics within this parasite–host–environment relationship can aid in the mapping of endemic areas that share similar profiles. ATL in RJ is apparently a benign disease, as severe forms, such as ML, are relatively rare.[9,10] Moreover, patients respond promptly to antimonial therapy, with even low doses being curative.[27] This can be explained by the particularities of the interactions between the host, vector, parasite, and environment that can affect the outcome of *Leishmania* infection. Therefore, a better understanding of the behavior of leishmaniasis in distinct regions of *L.* (*V.*) *braziliensis* transmission, such as Paraty, the second most important endemic area in RJ, is crucial in order to implement more efficient public health programs.

The highest incidence of cases in Paraty occurred amongst inhabitants from areas close to the rainforest. There was no preference with regard to gender, as described previously,[9,28,29] which refutes any relationship between infection risk and occupational activity. The ATL cases described in this study occurred predominantly in the first and second decades of life (45%); these values were lower than those observed in other areas of RJ.[10,12] The shift in age prevalence to the young, notably preschool children and infants, and the prevalence of facial lesions in this group support the role of households as a focus of infection. Moreover, it reflects a change in the epidemiologic and demographic patterns, with greater intra-domicile and peri-domicile transmission than rainforest exposure.[9,23]

The main clinical characteristic observed was a single cutaneous ulcerated lesion located in exposed areas of the body, similar to descriptions from other endemic areas.[9,10,12,28,29] The disease had a benign clinical course, and antimonial therapy was curative in most cases (data not shown). Two patients presented with multiple disseminated lesions associated with *L.* (*V.*) *braziliensis* infection, which constitutes a rare clinical form. The occurrence of DissL caused by *L.* (*V.*) *braziliensis* has been recorded in RJ,[9,30,31] although clinical descriptions of these cases have not been reported previously. An increasing number of cases of DissL have been documented in other Brazilian states, confirming that the clinical evolution differs from anergic DCL caused by *L.* (*L.*) *amazonensis*.[23,32–34] The multiple skin lesions may display a mixture of aspects, such as ulcerated, papular, nodular, and acneiform, with normally a few or no parasites visualized within affected tissues. The involvement of mucosal sites of the upper respiratory tract is also very common.[32] The patients described here showed a weak cellular response (positive MST but negative LPR to leishmanial antigens), but this clinical form is not always associated with a low parasite-specific immune response.[3,32] Both patients achieved cure (data not shown), even though one was resistant to antimonial therapy. In this specific case, the lesions worsened progressively, and clinical remission was obtained by a combination of antimonial plus immunotherapy.[17] These clinical and parasitologic characteristics provide further evidence for the difference between DissL and DCL.

In Paraty, 78% of the patients with active lesions were diagnosed within the first 3 months after the onset of the symptoms, as reported in surveys conducted in different endemic areas in RJ[9] and in other Brazilian states.[21,23] Notably, ML was not diagnosed in Paraty, corroborating the argument that this clinical form is not as frequent in RJ[9,10] as in other Brazilian regions.[20] No cases of ML were seen after a 10-year follow-up, and only five patients reported the relapse of CL during this period. Rapid and efficient diagnosis is important, and prompt treatment can decrease the probability of the disease evolving into more severe clinical forms. Nevertheless, we cannot exclude the possibility that the intrinsic genotypic characteristics of the circulating parasites[11] may account for the benign nature of the disease in RJ, in comparison with other Brazilian localities.

MST and serology were carried out in 88% and 100% of cases, respectively, and constitute the laboratory methods most frequently utilized by clinicians in Paraty. MST positivity (92% of patients) was in accordance with values described in other areas of RJ (93–97.9% positivity).[9,28] The serological diagnosis was positive in 56.3% of 71 patients tested. Although these values are expected for RJ,[18] the positivity rate for leishmaniasis by indirect immunofluorescence test was lower than that recorded in other regions.[21]

Lymphocytes and plasmocytes were the predominant cells in lesion infiltrates. Incomplete or organized granulomas were observed, compatible with histopathologic descriptions in Brazilian ATL patients.[35] Parasites were detected in the lesions in only two cases, as expected, as *L.* (*V.*) *braziliensis* amastigotes are sparse in lesions in comparison with *L.* (*L.*) *amazonensis* or *L.* (*V.*) *guyanensis* infection.[22,34] The rate of parasite isolation by axenic culture from skin was higher (78.5%) than that reported by other authors,[16,23] although this method was feasible in only 40% of patients.

Conventional parasitologic methods are known to confirm leishmanial etiology in around 60–80% of cases;[19,21,22] however, PCR methodology has improved the diagnosis by identifying up to 97% of CL cases caused by the subgenera *Viannia*.[19] In this study, patients in whom conventional methods did not confirm parasite infection had their skin tissues tested for *Leishmania* DNA detection. PCR was positive in most cases analyzed, and afforded subgenera identification in two cases with negative parasite cultures. As expected, PCR methodology was more sensitive and very useful in improving the confirmation of cases.

Leishmania (*V.*) *braziliensis* was the species characterized in all the samples analyzed, including the DissL patient. This

finding corroborates the proposal that this species is the most prevalent parasite in humans in RJ,[5,9] although *L.* (*L.*) *amazonensis* has recently been reported in one case from Paraty.[15] The identification of a predominant parasite species in a particular region is crucial not only from an epidemiologic point of view, but also for the establishment of the best treatment schedule and for clinical prognosis.

Conclusion

ATL in Paraty is an endemic infection in which benign cutaneous lesions caused by *L. braziliensis* predominate. Healthcare access and early diagnosis are probably important factors accounting for the favorable clinical course of the disease in this region. Although ML was not observed during the period of study (1991–2006), severe clinical forms of DissL were diagnosed in two patients. The disease in Paraty shares clinical and laboratory characteristics reported in other regions of RJ, probably because of the similar epidemiologic context related to the Atlantic rainforest region. These results contribute to the understanding of ATL in RJ, Brazil, help clinicians involved in the management of this disease in Paraty, and provide further information for public health program organization and leishmaniasis control.

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