Report

American tegumentary leishmaniasis (ATL) in Rio de Janeiro State, Brazil: main clinical and epidemiologic characteristics

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Abstract

Background Rio de Janeiro State in Brazil is an endemic area of American tegumentary leishmaniasis (ATL) induced by *Leishmania* (*Viannia*) *braziliensis*.

Objective Our purpose was to describe the main clinical and epidemiologic characteristics of the disease in Rio de Janeiro State.

Methods Patients from endemic areas of Rio de Janeiro State attending the Evandro Chagas Hospital were included in the study. A general physical, dermatologic, and otorhinolaryngologic examination was performed in all patients, as well as a Leishmanin skin test. Skin biopsy specimens were obtained and utilized for touch preparations (stained with Leishman dye), culture in special media (Nicolle, Nevy and McNeal; NNN), and histopathologic examination after hematoxylin and eosin stain. Positive cultures were identified with regard to species by the isoenzyme technique. Therapy with pentavalent antimonial compounds was employed in all cases. Eco-epidemiologic characteristics were studied through regular field visits to endemic foci.

Results Cutaneous disease was present in 87.2% of patients, and mucosal disease in only 12.7%. A single ulcerative cutaneous lesion was the most common clinical presentation. Demonstration of the parasite was always difficult and culture in special media gave the best results for diagnosis. The species involved in transmission was *Leishmania* (*Viannia*) *braziliensis*. Vectors included phlebotomine sand flies (Diptera: Psychodidae) of the genus *Lutzomyia*, and the most common species was *Lutzomyia intermedia*, captured mainly on the external walls of houses.

Conclusions ATL in Rio de Janeiro is mostly a cutaneous disease. In general, the cases showed great sensitivity to antimony. A pattern of peridomestic transmission seems to be the rule.

Introduction

Leishmaniasis (cutaneous, mucocutaneous, and visceral) occurs worldwide. According to the last report from the World Health Organization (WHO),[1] this disease is endemic in tropical and subtropical regions of 88 countries. American tegumentary leishmaniasis (ATL) is an important disease in Latin America. In Brazil, where the disease represents a public health problem,[2] ATL shows two distinct epidemiologic patterns. In the northern region, it is a typical zoonosis, with well-defined wild reservoirs and vectors, while humans are an occasional host.[3] In the southeast region, infection is nowadays present in endemic foci where the primary forest no longer exists.[4] At present, a well-defined wild reservoir has not yet been detected in these areas, but domestic animals are often infected.[5–7] The disease is caused mainly by *Leishmania* (*Viannia*) *braziliensis*.[8] In Rio de Janeiro State, the majority of the first cases described proceeded from other endemic regions of the

country, suggesting an imported disease.[9,10] Nevertheless, autochthonous cases have been described since the early 1900s.[11,12]

In this report, we have studied the disease in Rio de Janeiro State through the records of patients attending the Evandro Chagas Hospital. The majority of the patients were referred here by health centers in the state. Other cases were detected during an active search on regular visits by the staff to nearby endemic foci. The aim is to describe the clinical characteristics of the disease, together with epidemiologic data from the foci inhabited by the patients.

Materials and methods

Patients, routine diagnostic procedures, and therapy

Between 1985 and 1996, a total of 753 patients with ATL were diagnosed and treated at the Evandro Chagas Hospital, Oswaldo Cruz Institute-Fiocruz, Rio de Janeiro. Patients were classified into three major clinical groups: (i) cutaneous leishmaniasis (CL) for patients presenting with skin lesions only; (ii) mucocutaneous leishmaniasis (MCL) for patients with concomitant skin and mucosal lesions; and (iii) mucosal leishmaniasis (ML) for patients with mucosal lesions with or without a past history of healed skin lesions.

Complete physical and dermatologic examination included upper respiratory/digestive mucosae examination using an optical endoscope (Storz, Mainz, Germany). All patients were examined for mucosal lesions regardless of complaints of mucosal symptoms. An anterior rhinoscopy procedure was always used for examination of the nasal cavities. Patients presenting with lesions of the oral mucosa were selected for endoscopic examination, searching for extension of the lesions to the pharynx or larynx.

Leishmanin skin test (LST), biopsy of active lesions, and serologic tests were used as the routine diagnostic procedures. Skin test was performed in 752 patients according to the usual techniques. Leishmanin was provided by the Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais.[13] Reactions were considered to be positive when the induration area was equal to or greater than 5 mm. Incisional skin biopsy was performed after local anesthesia (lidocaine 2%) at the border of active lesions. One fragment was used in Nicolle, Nevy, and McNeal (NNN) medium and one was fixed in 10% buffered formalin, paraffin embedded and stained with hematoxylin and eosin (720 patients). Before fixing, a touch preparation was performed on two slides (710 patients) and stained with Leishman dye (Merck, Darmstadt, Germany).

An indirect immunofluorescence test was performed (332 patients) in order to detect anti-*Leishmania* antibodies.[14] Promastigote Brazilian strain MHCM/BR/76/JOF, phenotypically similar to *Leishmania major*,[15] was used as antigen. Antibodies titers of 1 : 45 or higher were considered to be positive. Fluorescence was evaluated using an epifluorescence microscope (Axiophot-Zeiss, Germany).

All patients were treated with pentavalent antimony (Glucantime®, Rhodia Laboratories, Antony, France). Besides the classical regimen of 20 mg/kg/day of Sb(V) during 3–4 weeks, alternative schedules (5 mg/kg/day and intralesional therapy) were also employed.

Identification of *Leishmania* species

The stocks isolated from patients were immunologically characterized and identified by the isoenzyme technique.[16] In a few doubtful cases, an indirect radioimmune binding assay or immunofluorescence technique, using species- and subspecies-specific monoclonal antibodies, was also employed.[17]

Epidemiology and ecology

Rio de Janeiro State is located in the southeast region of Brazil, facing the Atlantic. Geographically, the state is characterized by: (i) a region of coastal plains (the extensive Baixada Fluminense) intermingled with small massifs (Pedra Branca, Tijuca, Gericinó, and Niteroi) with altitudes up to 1000 m; (ii) the mountain ridge of Serra do Mar, located in the central inland region of the state, with altitudes up to 2000 m in a southwest–northeast orientation; and (iii) to the west, bordering the neighboring state of Minas Gerais, the Paraíba do Sul river valley dominates the landscape. In these three geographical areas, the remnants of the Atlantic forest may still be noted, along with secondary forests as well as nearby farming. All areas located in these geographical sites are potentially or currently endemic. The weather is hot and wet, with an annual average temperature of 18 °C in the coldest months. The state is divided into eight geopolitical regions, with a total population of 13,064,296 and a slightly larger proportion of females. Urban populations are largely predominant (92.25%).[18]

An active search was performed in five areas from where the majority of the studied cases originated (Mesquita, Campo Grande, Bangú, Realengo, and Magé). All of these areas are in the city of Rio de Janeiro metropolitan area. At least two visits per year were performed to each area during the period of the study.

Entomologic studies

Vectors were studied through weekly captures between 6 p.m. and 9 p.m using human and domestic animal baits or light traps.[19,20] Insects were collected inside and outside the houses. In the latter case, collection was performed separately in areas 50 m distant from the houses, between 50 and 100 m, and more than 100 m from the houses.

Statistical methods

A chart review was performed, retrieving some of the epidemiologic and clinical experience achieved during the period.

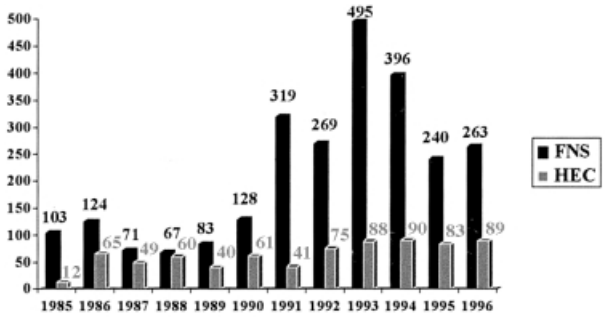

Figure 1 Reported cases of ATL in Rio de Janeiro State (all forms). Data from epidemiologic surveys by the National Foundation of Health (FNS) (black) and Evandro Chagas Hospital (HEC) (gray). Notification of all forms of leishmaniasis is compulsory in Brazil. As shown, HEC cases represent 29% of all reported cases in the state. The increase in the number of notified cases since 1991 is a reflection of the improvement of public health services in Brazil. *x*-axis, years; *y*-axis, number of cases

A simple description of variables was performed for the different clinical forms according to race, gender, age, residence, place of birth, lesion characterization (area, number of affected sites, anatomical region, lymphatic involvement), laboratory data, evolution, and outcome. Whenever applicable, analysis of variance (ANOVA), chi square for linear trends, or chi square (Yates) was used to measure the effect of the variables on the measures of immune response to *Leishmania*.

Results

Clinical forms

CL was present in 658 individuals (87.2%), MCL in 26 (3.5%), and ML in 69 (9.2%). Given that our data originate mainly from an outpatient clinic, many unavailable distorting biases (selection, measurement, etc.) are expected. As the notification of leishmaniasis is compulsory in Brazil and our data represent 29% of the officially reported cases (Fig. 1), however, we may infer that these biases do not affect our analysis, and that our data are representative of the disease in Rio de Janeiro State. In the Evandro Chagas Hospital, we have an average frequency of 63 cases per year.

Sex and age distribution

Our data show a men to women ratio of 1.6 : 1, but there is no statistical difference between ATL groups and gender. The disease is present in all age groups, but the second, third, and fourth decades of life are more frequently affected. Children under 10 years and elderly people over 60 years account for 11.7% and 11.4% of cases, respectively.

Profession

The majority of the population has professions unconnected to the land or forest. Profession is defined here as the main work activity of each individual at the time of appearance of the first lesion. Eighty-three per cent of all professions are typically urban, such as housekeeping, banking, industry, construction, hairdressing, etc. Only 17% of cases have professions related to rural activities or involving continuous exposure to a forest environment.

Cutaneous disease

Cutaneous lesions are shown in Fig. 2a–c. The predominant type of skin lesion was an ulcer (652 of 684 patients, 95.3%) and only a few patients presented with other types of lesion. The clinical aspects of the skin lesions in CL and MCL cases were similar but, while most CL cases had only one lesion (67.2%), MCL patients had two or more skin lesions in 92.3% of cases. In addition, skin lesions of CL were larger in size than those of MCL (Table 1). Cutaneous lesions were seen on the lower (40.7%) and upper (28.6%) extremities, face and neck (20.3%), and trunk (10.3%). Lymphatic involvement in CL and MCL was noted in 387 of 641 cases (60.4%). Considering only MCL cases, this involvement was even more frequent (19 of 26 cases or 73.0%). A striking difference concerning the duration of the disease was found among the three clinical forms (Table 2).

Mucosal disease

The majority of mucosal cases had an initial skin lesion when living outside Rio de Janeiro (60.9%, 42 of 69 cases), and statistically significant results were found with regard to the relationship between prior residence outside Rio de Janeiro and ML ($P < 0.0001$). The majority of cases (88%, 61 of 69) did not receive any treatment for the initial cutaneous lesion. In most ML cases, only one mucosal site was affected (42 of 69, 60.9%) and in 27 cases (39%) two or more sites were compromised. The nasal mucosa was the predominant site in 91.3% (63 of 69 cases). Clinically, nasal mucosa lesions were characterized mainly by diffuse infiltration, often associated with extension of the lesions to nasal skin and ulceration, followed by destruction of the nasal septum (43 of 63 cases, 68.2%), leading to the characteristic "tapir nose." The palate, the second most frequent mucosal location, usually showed a diffuse infiltration with a granular aspect (Fig. 2d–f).

Mucocutaneous disease

Contrary to mucosal disease, the majority of mucocutaneous cases arose from Rio de Janeiro State. The

Figure 2 (a) A single ulcer with raised borders was, by far, the most common clinical presentation. (b) Vegetating lesions were rare. (c) Lymph node involvement was noted in 60% of cases. (d) Lesions of the nasal mucosa extended to the skin of the upper lip. There was an increase in the volume of the nose and the beginning of the destruction of the subseptum. (e) Endoscopic aspect of the nose. The inferior cornet was increased and blocked the air passage, leading to nasal obstruction, a frequent complaint of patients. (f) Extensive lesion of the palate with destruction of the uvula

distribution of mucosal lesions was slightly different from ML cases, but the nasal mucosa was again the predominant site (21 of 26 cases, 80.7%). Two or more mucosal sites were commonly affected in MCL cases (14 of 26, 53.8%). Clinically, the lesions of ML and MCL cases were similar, although perforation of the nasal septum was more rarely seen in MCL cases (10 of 26, or 38.4%) probably due to its shorter duration (see Table 2).

Laboratory findings

Table 3 displays the laboratory findings in ATL groups. All laboratory data, except LST, differed among ATL groups. The most effective method to demonstrate parasites in all ATL groups was culture. In 30% of patients, parasitologic diagnosis was not possible with the three methods employed: in-prints, histopathology, and culture. Histopathology, even in the absence of the demonstration of parasites, was highly suggestive, although not diagnostic: there was a heavy, focal, inflammatory infiltrate composed mainly of lymphocytes, plasma cells, and macrophages. Early granuloma formation, fibrinoid necrosis, and frequent images of vasculitis made up the pathologic picture.

Table 1 Distribution of the number of skin lesions according to the clinical form

	CL	ML
No. of cutaneous lesions (mean)	1.9 ± 2.7	2.6 ± 1.1
No. of cutaneous lesions (median)	1.0	2.0
No. of cases with unique lesions*	442.0	2.0
No. of cases with more than one lesion*	216.0	24.0
Mean size of lesions (cm^2)†	5.72 ± 6.39	3.47 ± 6.21
No. of cases (total)	658	26

*χ^2 test, $P < 0.001$.
†F test, $P < 0.001$.

Identification of *Leishmania* species

One hundred and twenty-nine isolates were classified with regard to species: 128 were identified as *Leishmania*

(*Viannia*) *braziliensis* and one case as *Leishmania* (*Leishmania*) *chagasi*, causing a cutaneous ulcer.[21]

Therapy

Approximately 84% of patients were responsive to antimony therapy, achieving clinical cure in 25–45 days independent of the schedules used for therapy. At the Evandro Chagas Hospital, therapy with a low dose of antimony (5 mg/kg/day, intramuscularly applied during a 30-day period) for cutaneous[22–24] as well as for mucosal disease (manuscript in preparation) has proved to be equally effective as the high dose recommended by WHO (20 mg/kg/day). This therapy schedule has the additional advantage of presenting a low frequency of side-effects. In selected cases, such as pregnant women, elderly people, and patients suffering from severe cardiac or renal disease, intralesional therapy (for CL cases) with antimony was applied with good results.[25]

Epidemiology and ecology

In the studied foci, ATL was mostly found among the population living in the slopes and hillsides of the coastal massifs (localities of Mesquita, Campo Grande, Bangú, and Realengo) and the foothills of the central ridge of Serra do Mar (locality of Magé). In all these areas, human settlements were established after profound alteration of the original forest environment, a process that began many years ago and is still occurring. Brick houses and shacks are located alongside steep trails towards hilltops, usually shadowed by dense vegetation and banana plantations. The presence of human cases as well as affected domestic animals, such as dogs and horses, was a common finding in all studied foci[6,26,27] (Fig. 3a–d).

Table 2 Duration of disease (months) for ATL patients attending the Evandro Chagas Hospital according to the clinical form

Clinical form	Mean	Median
Cutaneous (CL)	3.58 ± 4.0	3.0
Mucocutaneous (MCL)	19.2 ± 34.4	5.5
Mucosal (ML)	120.2 ± 166.9	36.0

CL vs. MCL, $P < 0.02$. MCL vs. ML, $P < 0.0001$. CL vs. ML, $P < 0.0001$.

Entomologic studies

Lutzomyia intermedia was the predominant species found in all studied foci. Sand flies were captured both indoors and outdoors, in close vicinity to the houses and swarming in chicken coops usually located in the backyards of the houses. The sand flies were also present at some distance from the dwellings and even in the forest located far from the houses.[28] Table 4 gives an example of the species captured during a 30-month period in one of the endemic foci (Mesquita). The table shows that *Lutzomyia intermedia* represents 87.3% of the captured female species (2149 of 2460) and that 89.5% (1924 of 2149) were found less than 50 m from the houses.

Discussion

Rio de Janeiro State is an endemic area of *Leishmania* (*Viannia*) *braziliensis* infection, where secondary forest and agricultural land have replaced the original vegetation. Whenever human cases were found, dogs and horses were frequently affected. In general, patients performed urban occupations and only a small number practiced rural

Table 3 Laboratory diagnosis

	LST*	IFAT†	IP‡	H&E§	Culture¶
CL					
No. of cases	643/657	196/273	376/650	336/628	295/450
%	97.9	71.8	57.8	53.5	65.6
MCL					
No. of cases	26/26	17/18	17/24	19/25	16/21
%	100.0	94.4	70.8	76.0	76.2
ML					
No. of cases	69/69	36/41	ND	5/67	11/25
%	100.0	87.8	–	7.5	44.0

*LST, Leishmanin skin test; χ^2(Fisher), $P > 0.05$, any group combination.
†IFAT, immunofluorescence antibody test; χ^2(Fisher), $P < 0.05$, except for MCL vs. ML ($P > 0.05$).
‡IP, in-prints, Leishman stained; χ^2(Fisher), $P > 0.05$, any group combination; ND, not determined.
§H&E, histopathologic slides stained with hematoxylin and eosin; χ^2(Yates), $P < 0.0001$.
¶Culture in NNN medium; CL vs. MCL, χ^2(Fisher), $P > 0.05$; ML vs. any other, χ^2(Yates), $P = 0.05$.

Figure 3 (a) Landscape of one endemic focus (Magé). Dense vegetation intermingled with extensive deforested areas in the foothills of the mountain ridge of Serra do Mar. (b) Lesion in the foreleg of a mule. (c) View of the suburb of Campo Grande, just 18 miles from Rio de Janeiro city center. Human cases were present in the four houses seen in this picture. (d) Cutaneous ulcer in the ear of a dog. In all foci, the presence of the disease in humans and domestic animals was very common

professions. These facts suggest that the epidemiologic pattern of leishmaniasis in Rio de Janeiro State is mainly domestic or peridomestic.[27,29,30] Such a change in the epidemiology has led some authors to suppose that leishmaniasis may evolve into an urban disease.[31] According to some authors, ATL occurs in ecological systems limited to small geographic areas where remaining forest can still maintain the parasite life cycle.[32] In the absence of a proven sylvan reservoir, the high frequency of infection in domestic animals has led some authors to infer that these animals could represent the actual reservoir for this species.[33] This theory is questionable, as canine disease is very similar to human leishmaniasis with few parasites confined to the lesions.[34] It is noteworthy that, in the studied endemic foci, human dwellings are mainly located at periurban sites, but close to forested areas. Although the professions of the major part of the population are linked to urban activities, suggesting that the infection would have occurred near home, a reduced number of individuals interact intermittently with the forest, mainly for hunting or in small agricultural settlements. Women and children often participate in the latter activity. Furthermore, some patients have mentioned the presence of sylvan animals (sloths and opossums) in their backyards, a fact confirmed during our field visits, suggesting the possible involvement of a sylvan cycle in the transmission.

Lutzomyia intermedia does not fulfill all the criteria[35,36] for the determination of a vector. Nevertheless, it has been extensively demonstrated that the frequency, anthropophilic behavior, and geographical distribution of this species are coincident with human disease, suggesting its putative role as a vector.[37–41]

Unlike other *Leishmania* infections in the Old World, *Leishmania* (*Viannia*) *braziliensis* is difficult to demonstrate due to the scarcity of parasites in the lesions. In general practice, the diagnosis is often established mainly by the presence of three parameters: (i) compatible epidemiologic history; (ii) clinical aspect of the lesion; and (iii) a positive LST. A fourth parameter may be considered: the histopathologic picture can be highly suggestive, even in the absence of amastigotes. In addition, histopathology can also eliminate other cutaneous or mucosal lesions that are clinically similar to leishmaniasis, such as basal/epidermoid carcinomas or paracoccidioidomycosis. It is important to note that the parasitologic diagnosis of leishmaniasis has been improved considerably by more recent diagnostic techniques, such as the demonstration of *Leishmania* DNA by the polymerase chain reaction (PCR).[42]

Cutaneous disease, usually a single ulcerative lesion in exposed areas of the body, with frequent figures of lymph node enlargement and/or lymphangitis, is the most common

Table 4 Sand fly fauna in one endemic focus (Mesquita) in Rio de Janeiro State. Number of collected species during a 30-month period

	Indoors		Outdoors (<50 m)		50–100 m from house		800 m from house (forest)		Total	
Species	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**
B. guimaraes	–	–	1	3	1	1	–	–	2	4
Lu. longipalpis	10	14	28	202	3	–	–	–	41	216
Lu. migonei	11	10	176	650	3	5	3	2	193	667
Lu. lutziana	–	–	–	–	1	–	–	–	1	–
Lu. barretoi	–	–	–	1	–	4	–	–	–	5
Lu. intermedia	52	42	1924	1838	137	217	36	83	2149	2180
Lu. whitmani	–	–	–	–	1	1	–	–	1	1
Lu. schreiberi	–	–	–	2	–	2	–	–	–	4
Lu. quinquefer	17	3	44	8	1	–	2	–	64	11
Lu. sp.	–	–	7	–	2	–	–	–	9	–
Total	90	69	2180	2722	149	230	41	85	2460	3088
Total (M + F)	159		4902		379		126		5548	

M, male; F, female.

form of ATL in this part of Brazil. Cutaneous lesions are very sensitive to antimonial therapy. Spontaneous healing may be noted, but the lesions usually take a long time for complete cicatrization to occur (6 months to 1 year or more), as stated by several authors[43–46] and demonstrated by our own observations in 12 patients of this series.

Mucosal disease is rare. Precise data on the frequency of mucosal disease in Rio de Janeiro State are lacking. Early reports showed a variable prevalence, ranging from 8% to 80%.[47–50] In more recent epidemiologic surveys on restricted endemic areas, a frequency of 2.5% was found.[51,52] In the present series, we observed 9.2% of ML and 3.5% of MCL cases. In the mucosal form, most patients present a past history of inadequate or absent treatment of the initial cutaneous lesions.[53] Of our 658 treated cases of CL, the late appearance of mucosal lesions was observed in only three (0.4%). It is probable that the high frequency of mucosal disease seen in the first half of the twentieth century was due to the lack of effective therapy.

Mucocutaneous disease should be considered as a distinct form, as the duration of the disease, the age of the patients, and the number of cutaneous lesions are clearly different from ML or CL. In MCL cases, the mucosal symptoms develop soon after the appearance of cutaneous lesions, and therefore the mucosal disease has a much shorter duration than that of ML (see Table 2). Patients with MCL are also younger. In our series, the mean age of MCL cases was 39.6 years (youngest, 2 years; oldest, 78 years) while, in ML cases, the mean age was 55.4 years (youngest, 14 years; oldest, 92 years). In MCL patients, the number of cutaneous lesions was greater compared with CL (see Table 1) and also lymphatic involvement was more pronounced. These characteristics suggest a more aggressive behavior. There are at least two possible explanations, which are not mutually exclusive: the disease could be associated with an inappropriate immune response of the host and/or to a particular parasite strain. In the latter case, the parasite strain may have a higher capacity to invade macrophages and metastasize. The appearance of disseminated cutaneous lesions with mucosal involvement has been reported to be caused by parasites bearing dermotropic zymodemes in cases of human immunodeficiency virus (HIV) and *Leishmania* coinfection.[54] Similar findings were also present in visceral disease associated with HIV.[55] The association of tegumentary leishmaniasis and acquired immunodeficiency syndrome (AIDS) is rare: only four cases in the present series. A review of the literature disclosed only 19 cases and, of these, 13 (68.4%) were MCL cases.[56]

In summary, our data demonstrate that ATL in Rio de Janeiro State is mostly a cutaneous disease, with a remarkable sensitivity to pentavalent antimony. Early treatment may be implicated in the low rate of mucosal spread. Although transmission of the disease may be associated with a suspected (but not yet proven) sylvan cycle, peridomiciliary transmission seems to be the rule.

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