Endemic Tegumentary Leishmaniasis in Brazil: Correlation between Level of Endemicity and Number of Cases of Mucosal Disease

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Abstract. The purpose of this study was to establish a correlation between the endemic level of tegumentary leishmaniasis in different regions of Brazil during 2002–2009 and the number of cases of mucosal or mucocutaneous leishmaniasis. The proportion of mucosal leishmaniasis was inversely correlated with prevalence of infection. In areas with a lower infection prevalence, the proportion of mucosal leishmaniasis increased ($P < 0.05$). The hypothesis of an Amazonian origin and dissemination through human migration is considered. Our results show that in regions with lower prevalence and endemically younger, the proportion of cases that evolve to the mucosal form is higher than in regions with higher prevalence and endemically older.

INTRODUCTION

Tegumentary leishmaniasis (TL) is endemic to all Federal Units of Brazil at different levels. Clinical manifestations are classified as cutaneous leishmaniasis (CL) and mucosal leishmaniasis (ML); ML is also reported as mucocutaneous leishmaniasis (MCL).[1] Besides high morbidity coefficients, TL has received attention because of its expansion into urban centers.[2,3] Local differences in relation to *Leishmania* species; vectors; and environmental, socioeconomic, and biological determinants involved can be determined. The possibility of infection is a function of the presence of Phlebotomineae, sources of infection for these vectors, and susceptible human and animal populations.[2,4–6] The focal characteristics of leishmaniasis transmission hinders a better comparison among their incidence coefficients.[2]

Factors related to immune response of patients and parasite genetics appear to play a role in clinical expression and prognosis. Studies have shown intraspecific heterogeneity in members of the subgenera *Viannia* and *Leishmania* and have contributed to a better understanding of the epidemiology of the disease in different disease-endemic areas.[7–10] However, the role of the diversity of the parasite and its evolutionary, epidemiologic, and clinical consequences is not well established. Different studies using polymorphic markers, in an attempt to define genetic profiles, and biochemical and molecular characteristics of parasite populations have failed to establish a correlation between these profiles and clinical expression of MCL.[10–15]

Leishmania (*Viannia*) *braziliensis*, one of seven dermotropic *Leishmania* species found in Brazil, is responsible for localized, multiple, or disseminated cutaneous lesions and for the secondary effects in the mucosa. This species is broadly distributed in South and Central America and is predominant outside the Amazon region of Brazil, including rural and peri-urban areas. Some studies report *L.* (*V.*) *braziliensis* in approximately 55% of the TL cases in the Amazon region and in approximately 95% of TL cases outside the Amazon region.[14,16,17]

Species causing TL include *L.* (*V.*) *guyanensis*, which is predominant north of the Amazon River, and in Guyana, Peru, Ecuador, and Venezuela, has a well-established enzootic cycle, causes localized and disseminated predominately cutaneous lesions; *L.* (*Leishmania*) *amazonensis*, which is found less frequently in all regions, is associated with sylvan enzootic foci, and is responsible for localized clinical manifestations and infrequent anergic diffuse cutaneous forms of TL; and *L.* (*V.*) *lainsoni*, *L.* (*V.*) *naiffi*, *L.* (*V.*) *shawi*, and *L. (V.) lindenbergui*, which are limited to the Amazon region and cause localized cutaneous lesions.[2,5,6]

Division of Brazil into regions was proposed by the Brazilian Geographic Institute in 1969 on the basis of natural features such as climate, topography, vegetation, and hydrography.[18] Current knowledge shows that in the North region and neighboring areas of the Northeast and Central-West regions, the Amazon biome, which covers 49.3% of Brazil, is the dominant feature. The rest of the Brazil has different types of biomes (e.g., White Forest, Savanna, and Atlantic Forest).[2,19,20]

Leishmania (*V.*) *braziliensis* and its variants constitute a single group that are genetically related and found in natural and human-modified biomes and transmitted by several species of sand flies of the genera *Psychodopigus* and *Lutzomyia*. Infections are characterized by chronicity, latency, and single or multiple ulcerated cutaneous lesions, which may undergo spontaneous healing, or be reactivated and cause late destruction of upper aerodigestive tract mucosa. Treatment for of the disease is long and has social and high financial costs.[1,3,21,22] Although natural enzootic cycles of this agent are not defined, transmission is expanding to environments modified by peridomestic cycles that involve sand flies; humans; domestic animals such as dogs, horses, and cats; and occasionally wild animals.[2,4,6]

Reporting of TL in Brazil is compulsory through the Injury Notification Information System (Sistema de Informação de Agravos de Notificação),[23] which uses a form to codify patient sex and age, clinical manifestations, and other information. During recent decades, the Ministry of Health has recorded approximately 25,000 cases per year and 8–18 cases/100,000 inhabitants.[1,3,21] Unfortunately, parasite species are not routinely identified because of high costs of the methods involved. In this study, two types of clinical manifestations were considered: cutaneous leishmaniasis (CL) and mucosal leishmaniasis

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(ML). This purpose of this study was to establish a correlation between the incidence of TL in different regions of Brazil and the number of cases of ML or MCL.

MATERIALS AND METHODS

Areas studied. All TL cases available in the Injury Notification Information System during 2002–2009 were analyzed.[12] Persons with TL in Brazil were grouped by regions (North, Northeast, Central-West, Southeast, and South) to identify distribution of the disease.

Persons with TL. Data analysis was conducted in two steps. Initially, TL annual prevalence (Pp) per 100,000 inhabitants was estimated in the five regions of Brazil. The variables clinical manifestation and age were then chosen to construct indicators per region and group all years. The proportion of persons with ML (MLP) in relation to total TL cases was calculated for each region. MLP in relation to TL in minimum and maximum age groups (< 15 years and ≥ 40 years) was also calculated. These calculations enabled estimating the ratio of ML between minimum and maximum age groups. This ratio (denominated) is referred to as the extreme proportion ratio (EPR). An EPR > 1 indicates that ML is more frequent in the older age group (≥ 40 years) than in the younger age group.

Statistical analysis. Prevalence was adjusted for the expected proportion infected with *L. (V.) braziliensis* in different regions (55% in the Amazon region, and 95% in regions outside the Amazon region) because this species is the predominant species in the regions outside the Amazon region, including rural or peri-urban areas and also because other species rarely cause mucosal symptoms (adjusted prevalence [Ppa]). Simple correlation analysis was used with the indicators Ppa, Pp, MLP, and EPR, and the model was adjusted accordingly. Regression analysis was conducted for specific forecasting by determining the correlation (r), R^2, adjusted R^2 coefficients, and standard error of the estimate. Statistical analysis was conducted by using SPSS version 16 software (SPSS Inc., Chicago, IL). Significance level was fixed at $P < 0.05$. Distribution of cases with TL showed no significant differences regarding sex of the patients. Therefore, this variable was not adjusted in the statistical model.

RESULTS

The total number of TL cases, Ppa, Pp, and MLP in Brazil during 2002–2009 is shown in Table 1. A constant decrease in TL prevalence coefficients per period was observed. However, MLP showed no significant change during the study period.

Values for Pp, MLP, and EPR for each region are shown in Table 2. The highest Pp and Ppa values were found for the North and Central-West regions of Brazil, and the low-est values were found for The South and Southeast regions. An inverse correlation between prevalence (Pp or Ppa) and MLP was observed in the North, Central-West, Southeast, and South regions. This finding was not observed in the Northeast region. With high Pp and Ppa values, MLP tended to be lower (North and Central-West regions) and *vice versa* (Southeast and South regions). In the South region, the occurrence of ML in patients ≥ 40 years of age was approximately four times more frequent than in persons < 15 years of age.

Initial analysis of data from all regions of Brazil showed a negative correlation, although it was not significant. Subsequent evaluations suggested accepting the construction of an estimate that minimized adjustment errors. As a result, it was necessary to remove data for the Northeast region. The negative value found for R indicates a decreasing association of MLP and EPR for higher prevalence (Pp or Ppa) values. Adjusted R^2 values in linear equation were –0.980 ($P < 0.01$), –0.938 ($P < 0.05$), and –0.960 ($P < 0.05$) for dependent variables EPR, MLP and an age ≥ 40 years, respectively. The proportion with the mucosal form increased in relation to the decrease in prevalence. Such an increment was exponential with non-adjusted prevalence levels (Figure 1).

DISCUSSION

It is estimated that 3–5% of patients with CL in the New World will show natural evolution of the disease to ML.[1,3,22,24,25] This estimate reinforces the idea of considering ML as secondary to the cutaneous form, even in cases where a skin lesion is not detected or is clinically manifested as self-limited.[2,3,21,22]

Genetic studies have shown a large similarity between *L. (V.) braziliensis* isolates from the Amazon region and those from the South region of Brazil,[26] large genetic variability of this parasite in the Amazon region, and less variability in other regions of the country.[27,28] Such findings lead us to believe in clonal dispersion of a one lineage and reinforce the hypothesis of the Amazon origin of TL in Brazil,[2] in which the North region would be the focus of this endemic disease with later spread to other regions (Figure 2). Such dissemination would be more dependent on displacement of the human host than on other possible reservoir animals or vector insects.

Tegumentary leishmaniasis may have been introduced in the South region of Brazil at the beginning of the 20th century when TL cases were identified among workers in the construction of railways and roads.[29] However, during the 1950s, 1960s, and part of the 1970s, TL nearly disappeared in the Southeast region (São Paulo, Minas Gerais, Espírito Santo, and Rio de Janeiro), probably because of the end of deforestation and the massive use of organochlorine insecticides with large residual action in intensive agriculture and public health campaigns against malaria and Chagas disease.[2] As a result, there was a

TABLE 1

Number of cases of tegumentary leishmaniasis, prevalence in each year per 100,000 inhabitants, and proportion of those with mucosal leishmaniasis, Brazil*

Characteristic	2002	2003	2004	2005	2006	2007	2008	2009
No. of TL cases*	31,648	32,608	30,140	28,100	23,583	22,556	21,430	16,841
Prevalence of TL, %	18.12	18.44	16.60	15.26	12.63	12.26	11.30	8.79
% with ML	6.5	7.2	6.3	6.8	7.2	6.3	6.2	6,1

*TL = tegumentary leishmaniasis; ML = mucosal leishmaniasis.

TABLE 2
Prevalence of mucosal leishmaniasis by patient age in different regions of Brazil*

	MLP, %					
Region	< 15 years of age	≥ 40 years of age	EPR	MLP	Pp	Ppa
North	6.1	10.8	1.8	6.8	53.9	29.6
Central West	5.3	13.2	2.4	7.8	27.2	25.8
Northeast	2.6	5.8	2.1	3.7	5.9	5.6
Southeast	4.1	16.1	3.9	10.6	4.4	4.2
South	4.2	18.1	4.3	12.3	3.3	3.1
Total	4.6	10.7	2.3	ND	ND	ND

*ML = mucosal leishmaniasis; EPR = extreme proportion ratio; Pp = average prevalence during 2002–2009 per 100,000 inhabitants; Ppa = average prevalence during 2002–2009 per 100,000 inhabitants adjusted for the proportion infected with *Leishmania* (*Viannia*) *braziliensis*; ND = not determined.

loss of immunity in the human population induced by exposure to bites of Phlebotominae, whose saliva, even of those not infected, is believed to produce protection against *Leishmania* infections.[30] In some states of the South region, TL was detected during the 1980s (Paraná), 1990s (Santa Catarina), and in 2000 (Rio Grande do Sul).[1]

Reemergence of TL in the Southeast and South regions during the 1970s and 1980s and its spread to urban areas coincide with the end of malaria and Chagas disease control, the reappearance of anthropophilic Plebotominae species in environments with vegetation, and the intense migratory movement associated with construction of roads and the gold mining cycle in the Amazon, and subsequent return of part of this population to their original areas of residence.[2] South and Southeast regions would then be those where the endemic disease appeared more recently when compared with Central-West and North regions.

Evidence of a negative or decreasing correlation between Ppa or Pp and MLP and the presence of a correlated EPR indicates that there is a larger proportion of TL cases with the cutaneous form evolving to the mucosal form in regions to which this disease is not endemic. This finding suggests a
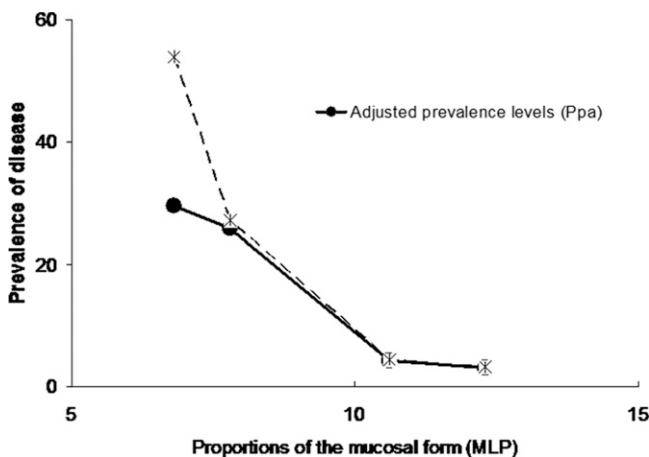
FIGURE 1. Results of nonlinear regression analysis between proportions of the mucosal form (MLP) and prevalence levels (Pp) or prevalence levels adjusted for the proportion of *Leishmania* (*Viannia*) *braziliensis* (Ppa). Shown is a summary of averages of all years in each region. The proportion of mucosal leishmaniasis appeared inversely related to the prevalence of infection. In areas of lower infection prevalence, the proportion of mucosal form increased. Such an increment was exponential with non-adjusted prevalence levels. The best fit model was R = −0.938, P < 0.05.

FIGURE 2. Theory of Amazonian expansion of tegumentary leishmaniasis by *Leishmania* (*Viannia*) *braziliensis*. Regions of Brazil are North, Central West, Northeast, Southeast, and South.

possible influence of the endemicity level in the form of the disease that is clinically manifested. The fact that there was a strong negative correlation (approximately −1) indicates the closeness of the points in relation to the linear equation of adjustment. The high R^2 value of −0.980 indicates that approximately 98% of EPR variance could be explained by adjusted prevalence level. Similarly, this finding was observed with MLP ($R^2 = −0.938$) and the MLP for persons ≥ 40 years of age ($R^2 = −0.960$).

By linking these observations to the theory of an Amazonian origin of TL[2] and other studies that that support this theory,[19,20,31–33] we can speculate on the parasite-host co-evolutionary mechanisms. Human populations in certain geographic areas may be more susceptible to *Leishmania* because of parasite introductions or reintroductions in more recent periods. However, in some regions, such interactions can be more complex because of epidemiologic overlapping (many introductions and reintroductions and old and new cycles), which may induce new epidemiologic patterns, as is the case of the Northeast region where a significant correlation between Ppa or Pp and MLP was not observed.

Furthermore, transmission in the Amazon areas and predominantly peri-domestic transmission in regions outside the Amazon region may have influenced our findings. The emergence of transmission foci depends on the Phlebotominae population growth in the primary peri-forest environment, immediately after deforestation (marginal effect) or on the fitness of certain vector species to rural or urban environments, which have been changed, and on possible sources of infection existent or introduced into these regions.

These suggestions challenge understanding of the factors related to clinical manifestation, including genetic diversity of species and subpopulations of parasites, vectors, and reservoirs, and genetic and immunologic factors of the host. The fitness of the parasite in different ecological conditions may also be associated with human migrations and consequent dispersion of the disease.[2,19] Intra-specific variability of TL

enables a *Leishmania* species to cause different clinical manifestations, with different severity levels, and different responses to treatment.[19] Because *L. (V.) braziliensis* predominates in all regions in different proportions, an advantage of this study is the adjustment of this variable in the model because this species is responsible for almost all mucosal disease.

Our hypothesis indicates that the magnitude of a disease endemic to a specific region and its history in that region produce fitness mechanisms for the interaction between the host and the parasite vector. This fitness would be able to reduce clinical manifestations of ML, which is considered a more severe form of TL. This suggestion would explain the larger proportion of TL cases evolving to ML in regions to which the disease has been endemic for a shorter time than in the North region, where the disease has been endemic for a longer time.

Studies of detection of parasites in cultures and its DNA in blood by polymerase chain reaction in the absence of active lesions,[34] in healed scars several years after treatment,[34–36] or in healthy skin before treatment[34] could explain reactivation of old lesions (induction through immunosuppression or injury) and spread to mucosa and non-apparent infections observed in disease-endemic areas. If over time the interaction mechanisms of the parasite with hosts and vectors lead to decreased manifestation of the more severe clinical forms of the disease, as occurs with urban CL in the Middle East, humans already considered a source of infection are likely to become a reservoir of *L. (V.) braziliensis*.[2,31,35] However, evidence that humans may be reservoirs of *L. (V.) braziliensis* is still limited to xenodiagnosis of Phlebotominae on the borders of skin lesions.[34]

Our findings show the need for further research to elucidate factors that modify clinical manifestations of the disease. This research will led to better understanding the role of the humans in maintenance of endemic leishmaniasis in Brazil and the importance of such findings to public health because of the continuous expansion of the disease in urban areas.

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