Trans R Soc Trop Med Hyg 2022; **116**: 70–79
https://doi.org/10.1093/trstmh/trab088 Advance Access publication 14 June 2021

ORIGINAL ARTICLE

Parasite species variation and impact of spatial displacement of the population on cutaneous leishmaniasis in Rio de Janeiro, Brazil

Mariza M. Salgueiro[a], Maria Inês F. Pimentel[a], Luciana F. C. Miranda[a], Rafael R. Cunha e Silva[b], Liliane F. A. Oliveira[a], Marcelo R. Lyra[a], Mauricio N. Saheki[a], Claudia M. Valete-Rosalino[a,c,d], Ana Cristina C. Martins[a], Armando O. Schubach[a,c], Mauro C. A. Marzochi[a,c], and Sandro J. Bedoya-Pacheco[b,*]

[a]Laboratório de Pesquisa Clínica e Vigilância em Leishmanioses, Instituto Nacional de Infectologia Evandro Chagas, Fundação Oswaldo Cruz, Av. Brasil, 4365, Manguinhos, Rio de Janeiro, CEP: 21040-900, RJ, Brazil; [b]Departamento de Epidemiologia e Métodos Quantitativos em Saúde, Escola Nacional de Saúde Pública Sérgio Arouca, Fundação Oswaldo Cruz, Rua Leopoldo Bulhões, 1480, Manguinhos, Rio de Janeiro, CEP: 21041-210, RJ, Brazil; [c]Productivity grant (PQ) of the "Programa de Produtividade em Pesquisa", Conselho Nacional de Desenvolvimento Científico e Tecnológico, SHIS QI 01, Conjunto B, Blocos A, B, C e D, Edifício Santos Dumont, Lago Sul, Brasília, CEP: 71.605-001, DF, Brazil; [d]Departamento de Otorrinolaringologia e Oftalmologia, Faculdade de Medicina, Universidade Federal do Rio de Janeiro, Av. Carlos Chagas Filho, 373 - Bloco K, 2° andar, Sala 49 - Cidade Universitária da Universidade Federal do Rio de Janeiro, Rio de Janeiro, CEP: 21044-020, RJ, Brazil

*Corresponding author: Tel: 55 21 3865 9541; E-mail: sandro.bedoya@ensp.fiocruz.br

Received 11 March 2020; revised 5 March 2021; editorial decision 26 May 2021; accepted 3 June 2021

Background: Cutaneous leishmaniasis results from complex interactions between human beings, vectors and the environment. Parasitic species differ in epidemiological and geographical contexts.

Methods: We studied a retrospective cohort of 696 patients with cutaneous leishmaniasis treated at a reference centre in the state of Rio de Janeiro, Brazil, between 2000 and 2015. We analysed displacements due to work, leisure and migrations with identification of *Leishmania* species.

Results: The geographic distribution of autochthonous cases showed that >95% of infections occurred in urban areas. In the state of Rio de Janeiro, most cases were concentrated in the cities surrounding forest parks and nature conservation areas. The same applies to the city of Rio de Janeiro, where these infections occurred in the neighbourhoods surrounding some mountain and forest areas. The non-displacement group included 575 (82.6%) patients and the displacement group included 121 (17.4%) patients. *Leishmania (Viannia) braziliensis* predominated in both groups. Other species were found in the displacement group.

Conclusions: The disordered urbanization of the state of Rio de Janeiro in recent decades has created conditions for the emergence of urban foci of transmission close to forest areas. Changes in the environment, movement of infected individuals and adaptation of sandflies may have contributed to this.

Keywords: Cutaneous Leishmaniasis, New World Leishmaniasis, Parasite Species, Epidemiology, Spatial Analysis

Introduction

Cutaneous leishmaniasis in Brazil has enormous diversity in the areas of transmission. In most regions where it is present, it results from the efficient adaptation of vectors to areas inhabited by humans or from the penetration of humans into areas inhabited by infected vectors. Most studies relate the disease to populations living in endemic areas, however, these areas have been expanding due to demographic and epidemiological dynamics.[1]

Socio-economic, cultural and environmental conditions contribute to the expansion of transmission areas. Exposure to the infectious vector followed by the development of cutaneous leishmaniasis may arise from professional and leisure activities. The movement of non-immune populations into areas where the disease is endemic is one of the subjacent causes.[2–5] There are situations in which individuals move to highly endemic areas due to working conditions. This has been recognized in the military contingents assigned to work in conflict regions or to training in jungle areas in various parts of the world.[6,7]

Occupations may increase the risk of exposure to infection, including agricultural and livestock activities,[8–10] geologists, biologists,[2,11] mining[12] and logging.[9] Deforestation associated with mining, logging, agriculture, livestock and road and railway construction may result in an increase in the disease.[13]

Leisure activities such as ecotourism, adventure tourism and mountaineering carry risks when they are performed in areas with the presence of infected vectors.[3,14]

Cases of cutaneous leishmaniasis in Rio de Janeiro were studied to assess the areas of transmission, professional activities and displacement due to migration, work and leisure. The species and variants of the parasite were identified.

Methods

Study design

We conducted a retrospective study of patients with cutaneous leishmaniasis treated at a reference centre in the state of Rio de Janeiro, Brazil, between 2000 and 2015.

Study population

All patients with cutaneous leishmaniasis treated between 2000 and 2015 at Evandro Chagas National Institute of Infectious Diseases (INI), Oswaldo Cruz Foundation (Fiocruz), Rio de Janeiro, entered the study. Patients who did not inhabit the state of Rio de Janeiro or cases with mucosal leishmaniasis were excluded. The study population was comprised of 696 patients. The study area was the state of Rio de Janeiro, which is located in southeastern Brazil. The state consists of 92 cities. All patients signed an informed consent form.

Clinical and epidemiological criteria included lesions (mainly ulcers) with infiltrated edges or infiltrated plaques. The protocol of laboratory diagnosis included parasitological exams (culture, direct exam, histopathological exam and kinetoplast DNA [kDNA] polymerase chain reaction [PCR]), serology (indirect immunofluorescence reaction and enzyme-linked immunosorbent assay) and Montenegro skin test. The direct examination consisted of the search for amastigotes in material obtained from skin lesions and arranged on slides through imprint (compression of a fragment of a lesion collected by a biopsy procedure) or smear technique. The diagnosis of cutaneous leishmaniasis was based on clinical characteristics and the positive result of culture, direct examination, histopathology or kDNA PCR.

Occupations and displacements due to work and leisure

Medical records from 696 patients were reviewed to obtain patient data such as age, sex, profession, home address, area of infection and information on travel. Professional activities were grouped according to the Ministry of Labor of Brazil. The category 'restricted to home' included children of preschool age, home workers, retirees and other situations that conditioned them to remain at home.

Regarding the area of transmission, patients were classified into four categories: residents in the state of Rio de Janeiro where the infection occurred (non-displacement group), infections due to displacement related to work, infections in immigrants from other states (imported cases) and infections due to leisure travel. The site of transmission of infection of the first group was defined by the residence. Regarding displaced groups, the site of transmission of infection was the area where this displacement occurred. Work and leisure displacements considered areas where the disease is highly endemic. An imported case was defined by the presence of a skin lesion before the arrival of an immigrant from another state.

Parasitic isolation in culture

Parasitic isolation in culture was performed on samples from 649 patients, according to the protocol registered at https://dx.doi.org/10.17504/protocols.io.22tggen. Briefly, fragments from a cutaneous lesion were cultured in Novy–MacNeal–Nicolle medium plus Schneider's Drosophila medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% foetal bovine serum and the antibiotics penicillin and streptomycin. Cultures for *Leishmania* were considered positive in optic microscopic analysis when mobile flagellated parasites with the characteristics of promastigote forms of the protozoa were visualized. Growth of promastigote cells was performed in appropriate sterile bottles until the parasites reached the stationary phase of growth. The parasites isolated in culture were cryopreserved in liquid nitrogen.

It was not possible to recover parasites from 173 (24.8%) samples after cryopreservation due to lack of growth and in 58 (8.3%) samples because of contamination by fungi or bacteria.

Aetiological identification of *Leishmania* species

Multilocus enzyme electrophoresis (MLEE)

Identification of species was determined preferably by MLEE, which is a biochemical characterization technique based on the pH-dependent electrophoretic mobility of a predefined set of proteins in a gel. The MLEE analysis was performed on 465 samples in which we recovered parasites. The total culture volume was centrifuged and the pellet was submitted to three washes in sodium chloride–ethylenediaminetetraacetic acid buffer under centrifugation to obtain the parasite mass for MLEE analysis. MLEE was performed on 1% agarose gel supported by Cal-Bond (124×258 mm; GE Healthcare, Chicago, IL, USA), using six or seven enzymatic systems: GPGDH (6-phosphogluconate dehydronegase, EC 1.1.1.43), GPI (glucose phosphate isomerase, EC 5.3.1.9), NH (nucleotidase, EC 3.2.2.1), G6PDH (glucose 6-phosphate dehydrogenase, EC 1.1.1.49), PGM (phosphoglucomutase, EC 5.4.2.2), ME (malic enzyme, EC 1.1.1.40) or MDH (malate dehydrogenase, EC 1.1.1.37). The MDH enzymatic system was used only when variant profiles were detected in the other enzymatic systems. Isoenzyme electrophoresis was performed with the reference strain of *Leishmania (Viannia) braziliensis* (MHOM/BR/M2903). If any sample presented a different profile, a new assay was performed with the other reference strains: *Leishmania (Leishmania) amazonensis* (IFLA/BR/19767/PH8), *Leishmania (Viannia) guyanensis* (MHOM/BR/1975/M4147), *Leishmania (Viannia) shawi* (MCED/BR/1984/M8408), *Leishmania (Viannia) lainsoni* (MHOM/BR/1981/M6426), *Leishmania (Viannia) naiffi* (MDAS/BR/1979/M5533) and *Leishmania (Leishmania) infantum* (MHOM/BR/1974/PP75). Analysis of gel bands was performed qualitatively by visual comparison of the sample band with the default reference strains.

PCR

Twelve samples with reduced growth in the culture and five that presented variant profiles in one or more enzymatic systems using the MLEE technique were also analysed by PCR–restriction fragment length polymorphism (RFLP) for species confirmation. DNA extraction was performed using a DNAzol Reagent Kit (Thermo Fisher Scientific, Waltham, MA, USA), following the manufacturer's recommendations. PCR assays were performed using the primers 5′GGACGAGATCGAGCGCATGGT3′ and 5′TCCTTCGACGCCTCCTGGTTG3′ to amplify a 234-bp fragment of the gene region encoding hsp70C. The amplification products were separated using 2% agarose gel electrophoresis with ethidium bromide (0.5 μg/mL) and visualized under ultraviolet light.

RFLP

Amplification products obtained by PCR were digested with two restriction enzymes, *Hae*III (Sigma-Aldrich) and *Bst*UI (Thermo Fisher Scientific), following the manufacturer's recommendations. The fragments obtained by enzymatic digestion were separated on a 12.5% polyacrylamide gel and stained with silver and bands were compared with a DNA fragment size marker (100-bp DNA ladder). The banding pattern was compared with the reference strains: *L. (V.) braziliensis* (MHOM/BR/1975/M2903), *L. (L.) amazonensis* (IFLA/BR/1967/PH8), *L. (V.) guyanensis* (MHOM/BR/1975/M4147), *L. (V.) shawi* (MCEB/BR/1984/M8408), *L. (V.) lainsoni* (MHOM/BR/1981/M6426) and *L. (V.) naiffi* (MDAS/BR/1979/M5533).

Sequencing

Sanger sequencing of the internal transcribed spacer of ribosomal DNA (ITS1-rDNA) was performed only in three cases where it was not possible to obtain taxonomic identification through MLEE or PCR-RFLP. The ITS1-rDNA was amplified by conventional PCR using the primers L5.8S: 5′-TGATACCACTTATCGCACTT-3′ and LITSR: 5′-CTGGATCATTTTCCGATG-3′. Amplification reactions were performed in volumes of 50 μL. Amplicons from the PCR-positive samples (300–350 bp, depending on the species) were visualized on a 2% agarose gel and purified using the Wizard SV Gel Kit and PCR Clean-up System Kit (Promega, Madison, WI, USA). The products were then sequenced with the same primers used in the PCR assay. Sequencing was performed on an automated sequencer at Plataforma de Sequenciamento Genômico ABI-3730 (Oswaldo Cruz Institute/Fiocruz).

Sequence alignment was performed using SeqMan Pro (DNASTAR, Madison, WI, USA) and comparisons were conducted with *Leishmania* reference strain sequences obtained from the GenBank database. Phylogenetics analyses with the evolutionary history were inferred using the maximum likelihood method based on the Jukes–Cantor model and the sequences were aligned using Molecular Evolutionary Genetic Analysis version 6 (Tokyo Metropolitan University, Tokyo, Japan; Arizona State University, Phoenix, AA, USA; King Abdulaziz University, Jeddah, Saudi Arabia). This same software was used to calculate a distance matrix and the genetic distance percentage between the test samples and reference strains of *Leishmania*.

Construction of maps

Maps were constructed using vector files from the state of Rio de Janeiro and its cities, available from the Brazilian Institute of Geography and Statistics (IBGE). The archives for the districts of the city of Rio de Janeiro were available from the Pereira Passos Institute. Both bases refer to the year 2015. With the construction and organization of the database, thematic maps were built using the open software Quantum GIS version 3.10.2. Thematic maps are intended to represent the spatial distribution of autochthonous cases in different cities in the state of Rio de Janeiro and the districts of the city of Rio de Janeiro. The classification of cities into predominantly urban, adjacent intermediate and adjacent rural was carried out according to the IBGE. The spatial distribution of the urban and rural cases of cutaneous leishmaniasis in the cities of the state of Rio de Janeiro, according to forest parks and nature conservation areas was represented in the adapted map of the State Institute of Environment for 2016 (http://www.inea.rj.gov.br/cs/groups/public/@inter_dibap/documents/document/zwew/mtiz/~edisp/inea0123058.pdf).

Statistic analysis

All data management and statistical analyses were performed using Excel 2013 (Microsoft, Redmond, WA, USA) and Statistical Package for Social Science (SPSS) for Windows, version 19.0 (IBM, Armonk, NY, USA). The tests were performed at the 5% level of significance.

Results

Occupations and displacements due to work and leisure

Of the 696 patients with cutaneous leishmaniasis, 257 (36.9%) were women and 439 (63.1%) men. Age ranged from 1 to 92 years (mean 35.9 y [standard deviation {SD} 19.2]). In women, the mean was 37.1 y (SD 20.7) and in men the mean was 35.2 y (SD 18.3). There were no significant differences between ages in relation to sex (p=0.17; 95% confidence interval −4964 to 0.911).

The geographic distribution of autochthonous cases showed that >95% of infections in the affected cities occurred in urban areas (Table 1 and Figure 1). During the 16 studied years, the territory of the state of Rio de Janeiro was progressively affected (Figure 2). Of the 92 cities in the state of Rio de Janeiro, cases of cutaneous leishmaniasis were found in 47, which corresponds to 22 563 km^2 (51%) of the territory (Figure 3A and Figure 4). In the state of Rio de Janeiro, most cases were concentrated in the cities surrounding the forest parks and nature conservation areas (Figure 4). The same applies to the city of Rio de Janeiro, where these infections occurred in the neighbourhoods surrounding some mountain and forest areas (Figure 3B and Figure 4).

The non-displaced group included 575 patients (82.6%) and the displaced group included 121 patients (17.4%). The first group had a great diversity of occupations, with a majority of

Table 1. Distribution of patients according to likely local of transmission of cutaneous leishmaniasis. Evandro Chagas Institute National Infectious, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, 2000–2015.

				Site of transmission, n (%)			
					Displacements (N=121 [17.4%])		
Occupations	Patients, n	Rural area, n (%)	Male, %	Residents in the area (N=575 [82.6%]	Work (n=52 [7.5%])	Migration (n=25 [3.6%])	Leisure (n=44 [6.3%])
Administrative	18	–	66.6	11 (1.9)	–	1 (4.0)	6 (13.6)
Farmer	58	51 (87.0)	91.3	53 (9.2)	2 (3.8)	1 (4.0)	2 (4.5)
Autonomous worker	8	–	87.5	8 (1.4)	–	–	–
Biologist	3	–	66.6	1 (0.2)	2 (3.8)	–	–
Cinematographer	1	–	100.0	–	1 (1.9)	–	–
Commerce	32	–	65.6	28 (4.9)	–	2 (8.0)	2 (4.5)
Construction	47	4 (8.5)	97.8	40 (7.0)	4 (7.7)	2 (8.0)	1 (2.3)
Drivers and highway workers	29	1 (3.4)	96.6	25 (4.3)	1 (1.9)	2 (8.0)	1 (2.3)
Environment analyst	1	–	100.0	–	1 (1.9)	–	–
Forest engineer	1	–	–	–	1 (1.9)	–	–
General services worker	96	6 (6.2)	59.3	86 (15.0)	3 (5.8)	3 (12.0)	4 (9.1)
Geologist	1	–	100.0	–	1 (1.9)	–	–
Geotechnical	1	–	100.0	–	1 (1.9)	–	–
Health professionals	8	1 (12.5)	12.5	5 (0.9)	2 (3.8)	-	1 (2.3)
Home-keeping	7	–	85.7	6 (1.0)	–	–	1 (2.3)
Industry worker	12	–	66.6	12 (2.1)	–	–	–
Military personnel	38	–	100.0	9 (1.6)	27 (51.9)	–	2 (4.5)
Miner	1	–	100.0	1 (0.2)	–	–	–
Mountaineer	1	–	100.0	–	1 (1.9)	–	–
Not reported	8	–	62.5	5 (0.9)	–	2 (8.0)	1 (2.3)
Oil technician	1	–	100.0	–	1 (1.9)	–	–
Police	2	–	100.0	1 (0.2)	–	–	1 (2.3)
Professionals	28	2 (7.1)	46.4	18 (3.1)	3 (5.8)	–	7 (15.9)
Quarry worker	1	–	100.0	1 (0.2)	–	–	–
Reforestation worker	2	1 (50.0)	100.0	1 (0.2)	1 (1.9)	–	–
Restricted to the house	150	14 (9.3)	24.6	137 (23.8)	–	9 (36.0)	4 (9.1)
Security worker	5	–	100.0	5 (0.9)	–	–	–
Students	135	11 (8.1)	63.7	121 (21.0)	–	3 (12.0)	11 (25.0)
Topographer	1	–	100.0	1 (0.2)	–	–	–
Total	696	91 (13.0)	63.0	575 (100.0)	52 (100.0)	25 (100.0)	44 (100.0)

individuals restricted to home (23.8%), students (21.0%), general service workers (15.0%) and farmers (9.2%). The second group included 52 (7.5%) patients with displacements for work reasons, 44 (6.3%) due to leisure travel and 25 (3.6%) infections in immigrants from other states (imported cases).

The work displacement group included mainly military personnel and work activities considered to be at higher risk of exposure to infection: biologist in fieldwork, environmental analyst, forest engineer, geologist, geotechnical worker, reforestation worker, oil technician, topographer and quarry worker. We also had a cinematographer and a professional mountaineer who work in endemic areas. Twenty-seven of the 38 military personnel were stationed in an endemic area and a part of them participated in training in the Amazonian jungle (Table 1).

Males predominated in most occupations in the displacement and non-displacement groups (Table 1).

Leishmania species typing

In 465 (66.8%) samples it was possible to characterize species of *Leishmania* and in five samples it was possible to determine genetic variants through MLEE (n=448), PCR-RFLP (n=14) and sequencing (n=3). *L. (V.) braziliensis* was the predominant species. Almost all infections where transmission occurred in areas of residence were due to *L. (V.) braziliensis*. In 12 samples, *L. (V.) naiffi*, *L. (V.) guyanensis* and *L. (L.) amazonensis* were found (Table 2). Most of the samples that were not *L. (V.) braziliensis* were associated with displacement due to work reasons in areas

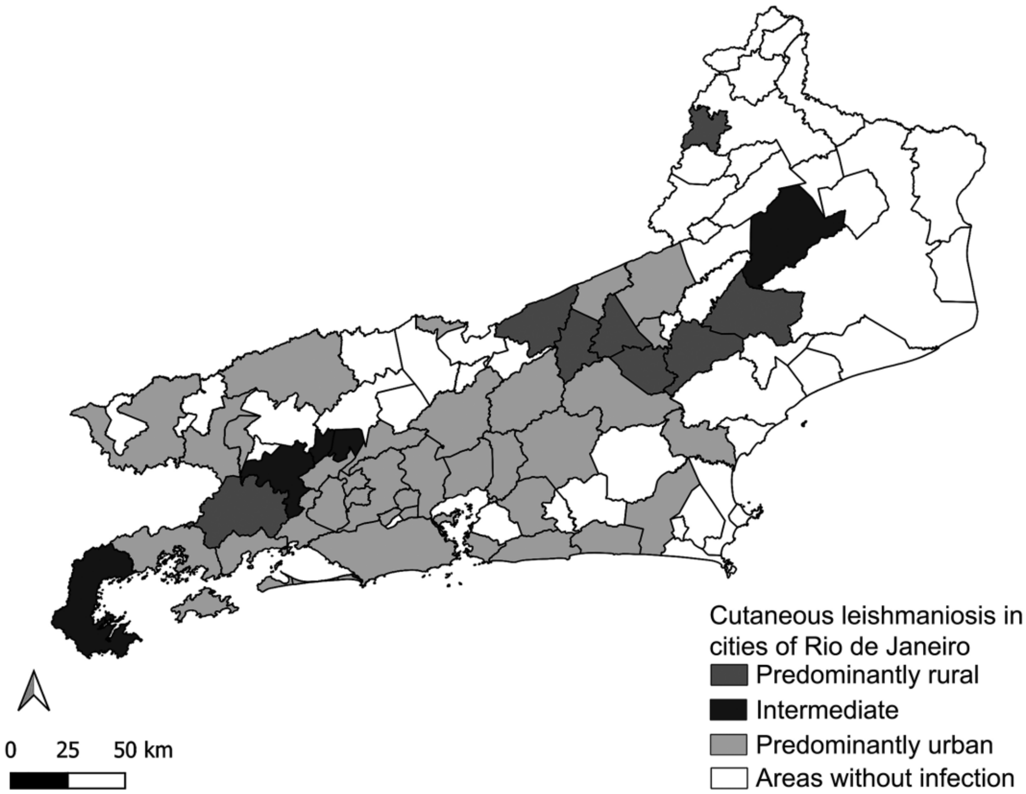

Figure 1. Distribution of the cases of cutaneous leishmaniasis in the cities of the state of Rio de Janeiro, according to transmission in urban, peri-urban and rural areas. Evandro Chagas National Institute of Infectious Diseases, Oswaldo Cruz Foundation (Fiocruz), Rio de Janeiro, Brazil, 2000–2015.

with the presence of sandfly vectors and reservoirs (13 of 16 samples), with military personnel representing 8 of those cases. A genetic variant of *L. (V.) braziliensis* was found in one of the individuals residing in Rio de Janeiro and in three imported cases in individuals who were infected due to working conditions. One genetic variant of *L. (V.) naiffi* was identified in the work displacement group.

Discussion

One of the gaps in the epidemiology of human infection with cutaneous leishmaniasis is related to the risk of transmission associated with human activities in each context. Our results show that in the state of Rio de Janeiro, most infections are autochthonous and occur in the areas where individuals live, while a much smaller proportion occur in conditions of displacement due to work, migration or leisure activities.

The extensive transmission area in the state of Rio de Janeiro was distributed predominantly in urban areas. In the past, the endemic disease in Brazil had a mainly rural character,[13] which still happens in parts of the north and northeast of the country.[8] Rio de Janeiro seems to resemble states in the south and southeast regions.[15]

Most of the cases in the state of Rio de Janeiro occurred in areas surrounded by forest or mountainous regions. Many of these parks are areas of that are progressively being transformed into urban areas. The same applies to the city of Rio de Janeiro, where these infections occurred in the neighbourhoods surrounding the mountains and forest parks. The historical trend of the disease in the city of Rio de Janeiro shows an increase in peripheral areas.[16]

The disordered urbanization of the state of Rio de Janeiro in recent decades has created conditions for the emergence of urban foci of transmission close to forest areas. This might be due to the movement of infected individuals to urban areas where the sandfly already existed or has adapted. It may be a change from the previous sylvatic transmission cycle to a new urbanized transmission cycle. The hypothesis of humans contributing to the dynamics of the new cycle of transmission should be considered.

Men predominated in most occupations, regardless of the displacement. A balanced distribution between sexes would be expected when the probability of transmission is homogeneous.[4] However, there are reports in the literature that suggest that men are more susceptible to the disease.[17] Infections due to occupational conditions may have specific proportions, as in military personnel and farmers, where males predominate.[8,10,15]

Farmers represented an important group and most lived close to their work areas. Historically, rural activities in areas with infected reservoirs and sandflies have been associated with

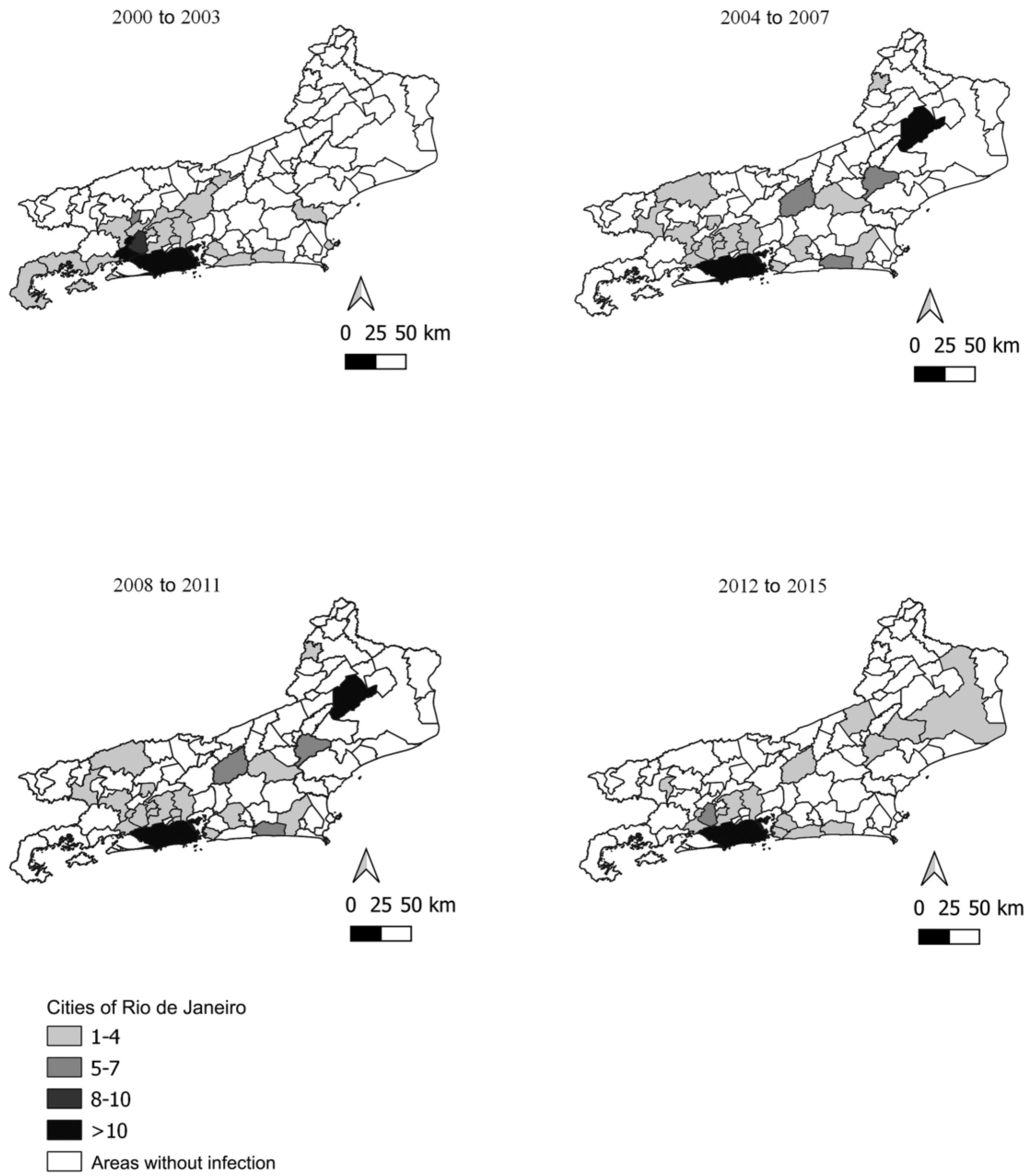

Figure 2. Distribution of the cases of cutaneous leishmaniasis in the cities of the state of Rio de Janeiro, according to the quadrennium of the occurrence. Evandro Chagas National Institute of Infectious Diseases, Oswaldo Cruz Foundation (Fiocruz), Rio de Janeiro, Brazil, 2000–2015.

cutaneous leishmaniasis.[18] Sandflies of different species have been found in coffee, sugar cane and fruit tree plantations and other agricultural areas.[19–21] Rural households should be built at an adequate distance from the cultivation sites.[20] The difficulty of accessing healthcare, the cost of transportation and the economic repercussions of interruption of work are important problems in this group. These difficulties translate into a late diagnosis with possible worsening of the condition. Perception studies of the disease carried out in a portion of these patients showed that the majority were unaware of the transmission mechanism.[22] How the affected individuals perceive the illness process and how health professionals identify the risk of transmission play a fundamental role in the development of efficient strategies for control and prevention.[22]

Military personnel accounted for the largest proportion of infections in the group of displacements due to work. Many of them are moved to endemic areas to perform exercises in the jungle. Previous studies have shown high proportions of the disease, reaching up to 25% of the exposed military personnel.[6,7,23,24] Protection measures, such as the use of long sleeves and bed nets treated with insecticide, should be used to reduce the risk of transmission in these populations.[25] The very nature of military training in the jungle often makes it difficult to properly use these measures,[14] which are not always effective,[23] possibly due

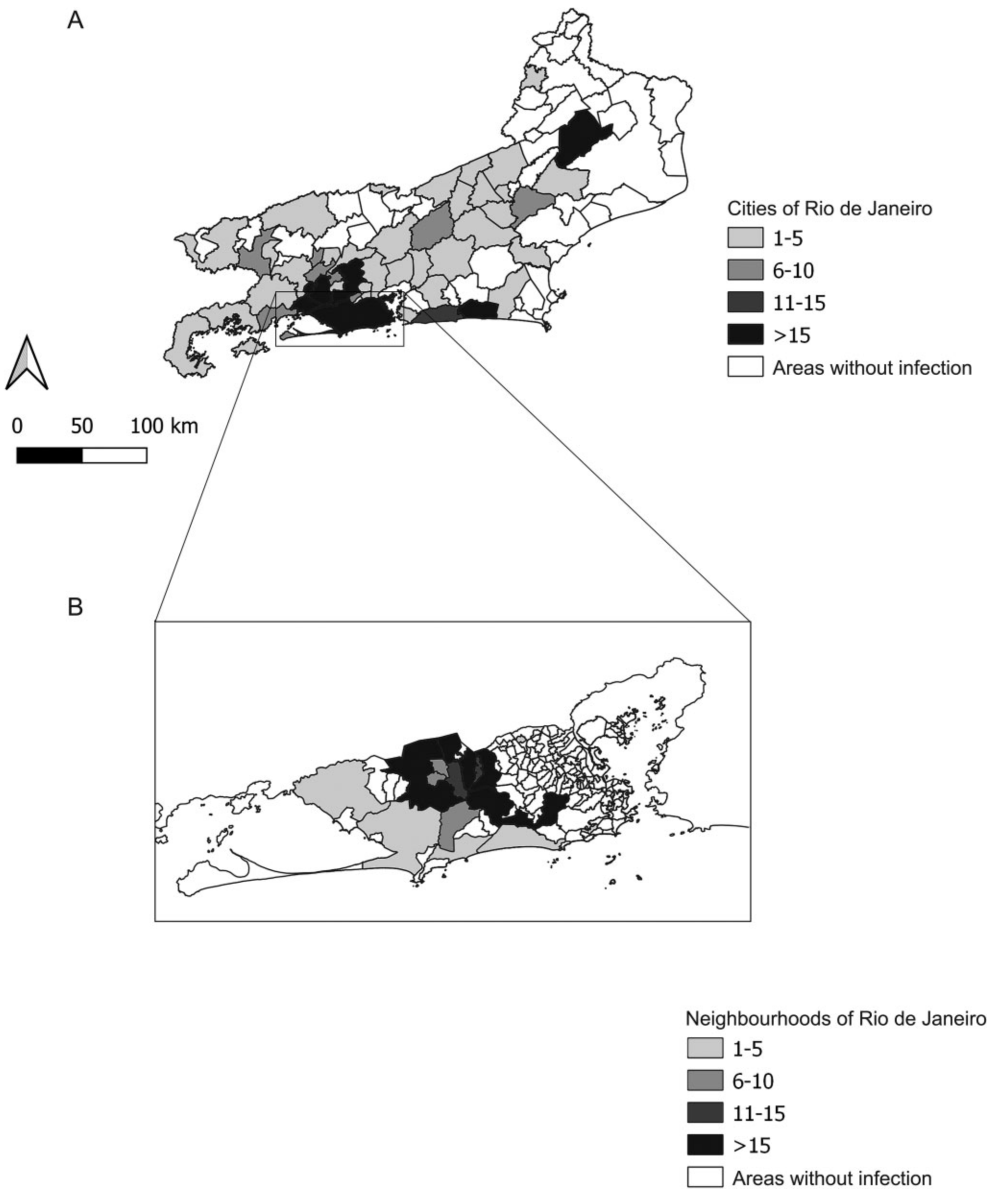

Figure 3. Distribution of the cases of cutaneous leishmaniasis according to the area of transmission in (**A**) cities of the state of Rio de Janeiro and (**B**) neighbourhoods of the city of Rio de Janeiro. Evandro Chagas National Institute of Infectious Diseases, Oswaldo Cruz Foundation (Fiocruz), Rio de Janeiro, Brazil, 2000–2015.

to permanent exposure. Entomological studies at military training bases in the Amazon have detected a great diversity of sandflies,[25] showing the risk of exposure to infection.[26]

We identified different occupations that are considered to be at risk because they increase the probability of transmission due to penetration in areas with a high concentration of infected vectors.[2,11] Surveillance actions, periodic assessments and provision of appropriate equipment are the responsibility of the companies, institutions and responsible authorities.

Our results reinforce the notion that cutaneous leishmaniasis is also a disease related to tourism and leisure. Almost 7% of our sample had illness related to travel to endemic areas. Ecotourism and adventure tourism in forest areas in Latin America have been highlighted as a risk and are included in recommendation guides for travellers.[3,5] Diagnosis of cutaneous leishmaniasis should be considered in tourists who return from endemic regions with lesions that do not heal. Once cutaneous leishmaniasis is confirmed, treatment can be directed by the geographical area of the origin of the infection, however, it is recommended that the species be identified and that the treatment should be directed to the aetiologic agent.[5] The availability of medications, the need for hospitalization and the recommendations of the referral centres must be taken into account. Counselling before travel is important to prevent the occurrence of disease,[27] particularly regarding the use of repellents and protective screens.

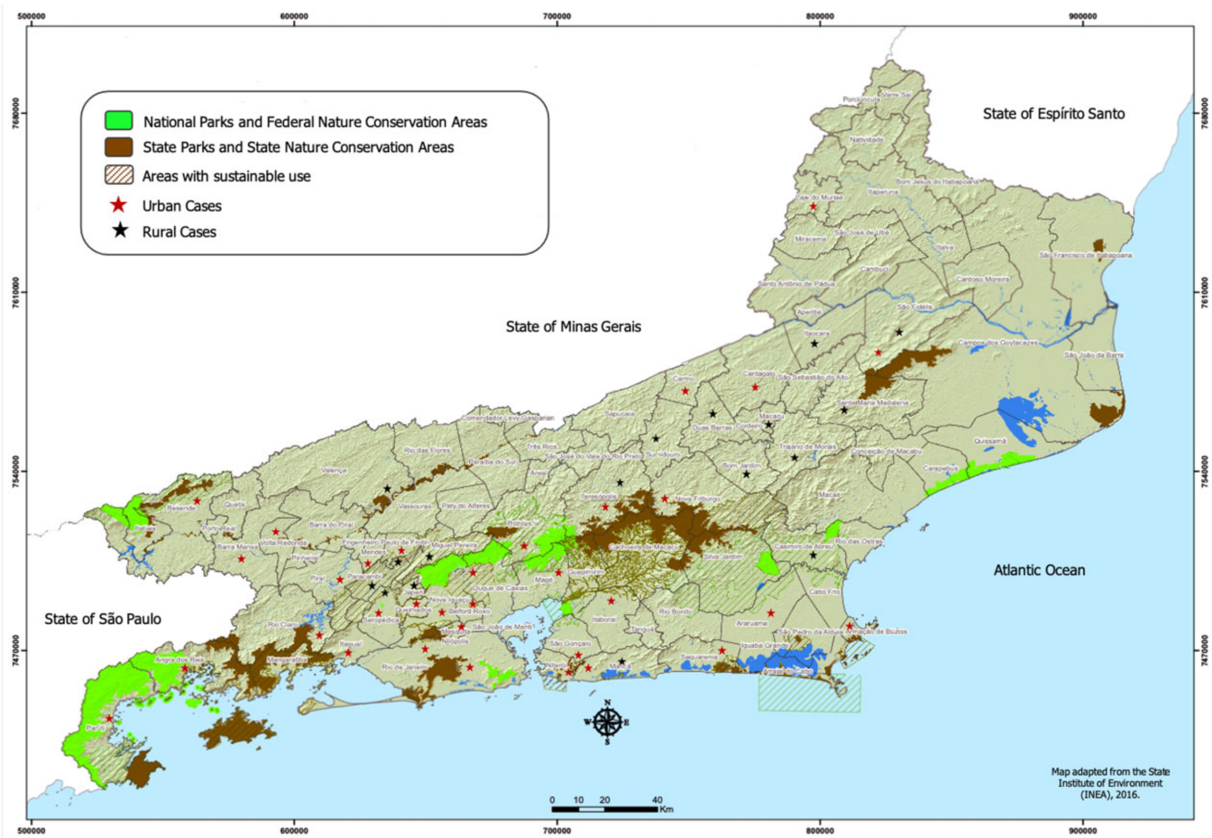

Figure 4. Spatial distribution of the urban and rural cases of cutaneous leishmaniasis in the cities of the state of Rio de Janeiro according to forest parks and nature conservation areas. Evandro Chagas National Institute of Infectious Diseases, Oswaldo Cruz Foundation (Fiocruz), Rio de Janeiro, Brazil, 2000–2015. Most cases are concentrated in the territories surrounding the forest parks and nature conservation areas.

Table 2. Distribution of *Leishmania* according to location of transmission. Evandro Chagas National Institute of Infectious Diseases, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, 2000–2015.

	Local of transmission, n (%)				
		Displacements (n=121 [17.4%])			
Parasite species	Residence (n=575 [82.6%])	Work (n=52 [7.5%])	Migration (n=25 [3.6%])	Leisure (n=44 [6.3%])	Total
L. (L.) amazonensis	–	1 (3.1)	–	–	1 (0.2)
L. (V.) braziliensis	400 (99.8)	19 (59.4)	15 (100.0)	15 (88.2)	449 (96.5)
L. (V.) braziliensis (variant)	1 (0.2)	3 (9.4)	–	–	4 (0.9)
L. (V.) guyanensis	–	3 (9.4)	–	2 (11.8)	5 (1.1)
L. naiffi	–	5 (15.6)	–	–	5 (1.1)
L. naiffi (variant)	–	1 (3.1)	–	–	1 (0.2)
Total	401 (100.0)	32 (100.0)	15 (100.0)	17 (100.0)	465 (100.0)
Uncharacterized proportion	174 (30.3)	20 (38.5)	10 (40.0)	27 (61.4)	231 (33.2)

Species other than *L. (V.) braziliensis* were only found in conditions of displacement, especially due to work, as in the case of military personnel who participated in training in the jungle. Although *L. (V.) braziliensis* is considered to be a genetically homogeneous species, its behaviour appears to differ between geographic regions.[28] Genetic studies of *Leishmania* populations show a great diversity of species and variants in the Amazon[29] and Northeast region.[30] *L. (V.) braziliensis* is largely prevalent in the Southeast region, including the state of Rio de Janeiro. We have identified variants of *L. (V.) braziliensis* and *L. (V.) naiffi* species. It is difficult to predict the impact of the introduced diversity and whether the new parasite species will adapt to the territory of Rio de Janeiro.

The main limitation of the study is the fact that the population treated at our centre represents 40% of infections in the state of Rio de Janeiro. We also had a high proportion of sample losses due to the absence of growth in culture.

Conclusions

The majority of cutaneous leishmaniasis infections in our study occurred in the areas where individuals live and a minority were due to displacement secondary to immigration, work and leisure activities. Populations established close to nature conservation areas and forest parks create conditions that favour transmission of the disease. Our results showed the epidemiological complexity of cutaneous leishmaniasis in the territory of Rio de Janeiro. It is necessary to develop spatial studies that consider the territorial dynamics of the vectors and population, taking into account the geographical, biological and social characteristics.

Authors' contributions: MMS, MIFP and SJBP conceived the study. MIFP and SJBP designed the study protocol, analysed and interpreted the data and drafted the manuscript. MMS, MIFP, LFCM and SJBP retrieved epidemiological and clinical data. RRCS performed geocodification. LFCM performed species characterization. All the authors critically revised the manuscript for intellectual content and read and approved the final manuscript. MIFP and SJBP are the guarantors of the paper.

Funding: This study was funded by Coordenação de Pessoal de Nível Superior (grant 001). It was also funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico (grants AOS 304335/2014-2, MCAM 308889/2017-7 and CMVR 313327/2018-1). The funders had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript.

Competing interests: None declared.

Ethical approval: This study was approved by the Research Ethics Committee of the Evandro Chagas National Institute of Infectious Diseases, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil (CAAE 61114116.4.0000.5262).

Data availability: Data are available upon reasonable request to the corresponding author.

References

1 Bedoya-Pacheco SJ, Araujo-Melo MH, Valete-Rosalino CM, et al. Endemic tegumentary leishmaniasis in Brazil: correlation between level of endemicity and number of cases of mucosal disease. Am J Trop Med Hyg. 2011;84(6):901–5.

2 Soares L, Abad-Franch F, Ferraz G. Epidemiology of cutaneous leishmaniasis in central Amazonia: a comparison of sex-biased incidence among rural settlers and field biologists. Trop Med Int Health. 2014;19(8):9888–95.

3 Mansueto P, Seidita A, Vitale G, et al. Leishmaniasis in travelers: a literature review. Travel Med Infect Dis. 2014;12(6 Pt A):563–81.

4 Monteiro WM, Neitzke-Abreu HC, Ferreira ME, et al. Population mobility and production of American tegumentary leishmaniasis in the state of Parana, southern Brazil. Rev Soc Bras Med Trop. 2009;42(5): 509–14.

5 Blum J, Buffet P, Visser L, et al. LeishMan recommendations for treatment of cutaneous and mucosal leishmaniasis in travelers, 2014. J Travel Med. 2014;21(2):116–29.

6 van Thiel PP, Zeegelaar JE, Van Gool T, et al. Cutaneous leishmaniasis in three Dutch military cohorts following jungle training in Belize. Travel Med Infect Dis. 2011;9(3):153–60.

7 Oré M, Sáenz E, Cabrera R, et al. Outbreak of cutaneous leishmaniasis in Peruvian military personnel undertaking training activities in the Amazon Basin, 2010. Am J Trop Med Hyg. 2015;93(2):340–6.

8 Araujo AR., Portela NC, Feitosa AP, et al. Risk factors associated with American cutaneous leishmaniasis in an endemic area of Brazil. Rev Inst Med Trop Sao Paulo. 2016;58:86.

9 Hernández AM, Gutierrez JD, Xiao Y, et al. Spatial epidemiology of cutaneous leishmaniasis in Colombia: socioeconomic and demographic factors associated with a growing epidemic. Trans R Soc Trop Med Hyg. 2019;113(9):560–8.

10 Eid D, Guzman-Rivero M, Rojas E, et al. Risk factors for cutaneous leishmaniasis in the rainforest of Bolivia: a cross-sectional study. Trop Med Health. 2018;46:9.

11 Brito ME, Andrade MS, Almeida EL, et al. Occupationally acquired American cutaneous leishmaniasis. Case Rep Dermatol Med. 2012;2012:279517.

12 Rotureau B, Joubert M, Clyti E, et al. Leishmaniasis among gold miners, French Guiana. Emerg Infect Dis. 2006;12(7):1169–70.

13 Temponi AOD, Brito MG, Ferraz ML, et al. Ocorrência de casos de leishmaniose tegumentar Americana: uma análise multivariada dos circuitos espaciais de produção, Minas Gerais, Brasil, 2007 a 2011. Cad Saúde Pública. 2018;34(2):e00165716.

14 Vanbrabant P, van den Broucke S, Soentjens P. Cutaneous ulcer after a stay in the tropics. Neth J Med. 2015;73:44.

15 Detoni MB, Lima DM, Silva TP, et al. Temporal and spatial distribution of American tegumentary leishmaniasis in north Paraná: 2010–2015. Rev Soc Bras Med Trop. 2019;52:e20180119.

16 Kawa H, Sabroza PC. Espacialização da leishmaniose tegumentar na cidade do Rio de Janeiro. Cad Saúde Publica. 2002;18(3): 853–65.

17 Lockard RD, Wilson ME, Rodriguez NE. Sex-related differences in immune response and symptomatic manifestations to infection with *Leishmania* species. J Immunol Res. 2019;2019:4103819.

18 Pedrosa FA, Ximenes RA. Sociodemographic and environmental risk factors for American cutaneous leishmaniasis (ACL) in the State of Alagoas, Brazil. Am J Trop Med Hyg. 2009;81(2):195–201.

19 Miranda DE, Sales KG, Faustino MA, et al. Ecology of sand flies in a low-density residential rural area, with mixed forest/agricultural exploitation, in north-eastern Brazil. Acta Trop. 2015;146: 89–94.

20 Moreno M, Guzmán-Rodriguez L, Valderrama-Ardila C, et al. Land use in relation to composition and abundance of phlebotomines (Diptera: Psychodidae) in five foci of domiciliar transmission of cutaneous leishmaniasis in the Andean region of Colombia. Acta Trop. 2020;203:105315.

21 Souza NA, Andrade-Coêlho CA, Vilela ML, et al. The Phlebotominae sand fly (Diptera: Psychodidae) fauna of two Atlantic rain forest reserves in the state of Rio de Janeiro, Brazil. Mem Inst Oswaldo Cruz. 2001;96(3):319–24.

22 Martins ACC, Nunes JA, Bedoya-Pacheco SJ, et al. Perception risk of transmission of zoonoses in a reference center. Rev Eletron Comun Inf Inov Saude. 2015;9(3):1–14.

23 Andrade MS, Brito MEF, Silva ST, et al. Leishmaniose tegumentar Americana causada por *Leishmania (Viannia) braziliensis* em área de treinamento militar na Zona da Mata de Pernambuco. Rev Soc Bras Med Trop. 2005;388(3):229–33.

24 Guerra JAO, Talhari S, Paes MG, et al. Aspectos clínicos e diagnósticos da leishmaniose tegumentar Americana em militares simultaneamente expostos à infecção na Amazônia. Rev Soc Bras Med Trop. 2003;36(5):587–90.

25 Lightburn E, Meynard JB, Morand JJ, et al. Surveillance épidémiologique des leishmanioses tégumentaires en Guyane. Synthése de données militaires sur 10 ans. Med Trop. 2002;62:545–53.

26 Gomes LHM, Albuquerque MIC, Rocha LC, et al. Diversity and distribution of sandflies (Diptera: Psychodidae: Phlebotominae) in a military area in the state of Amazonas, Brazil. Mem Inst Oswaldo Cruz. 2013;108(5):651–6.

27 Savoia D. Recent updates and perspectives on leishmaniasis. J Infect Dev Ctries. 2015;9(6):588–96.

28 Cupolillo E, Brahim LR, Toaldo CB, et al. Genetic polymorphism and molecular epidemiology of *Leishmania (Viannia) braziliensis* from different hosts and geographic areas in Brazil. J Clin Microbiol. 2003;41(7):3126–32.

29 Silva ACT, Cupolillo E, Volpini AC, et al. Species diversity causing human cutaneous leishmaniasis in Rio Branco, state of Acre, Brazil. Trop Med Int Health. 2006;11(9):1388–98.

30 Brito MEF, Andrade MS, Mendonça MG, et al. Species diversity of *Leishmania (Viannia)* parasites circulating in an endemic area for cutaneous leishmaniasis located in the Atlantic rainforest region of northeastern Brazil. Trop Med Int Health. 2009;14(10):1278–86.