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Flebotomíneos naturalmente infectados por *Leishmania* e fatores preditores de leishmaniose tegumentar americana no Brasil: uma revisão sistemática

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Co-orientadora: Prof. Jussara Kasuko Palmeiro

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LISTA DE ABREVIATURAS E SIGLAS

ATL	American tegumentary leishmaniasis
WHO	World Health Organization
IDRM	Montenegro's intradermal reaction
PRISMA	Systematic Reviews and Meta-Analysis checklist
PECOS	Population, exposition, comparator, outcome and study design
VL	Visceral leishmaniasis
DNA	Desoxyribonucleic acid
RNA	Ribonucleic acid
PCR	Polymerase Chain Reaction
RFLP	Restriction fragment length polymorphism
LnPCR	Leishmania-specific nested polymerase chain reaction
MLEE	Multilocus enzyme electrophoresis
MCAb	Indirect immunofluorescence method
KDNA	Kinetoplast DNA
hsp70	70 kilodalton heat shock proteins
ITS1	Internal transcribed spacer 1 region
SSUr DNA	Small subunit ribosomal DNA
SSUr RNA	Small subunit ribosomal ribonucleic acid
HgaI	Haemophilus gallinarum
SL RNA	Spliced Leader RNA
ITS rDNA	rDNA Transcript Internal Space
L.	*Leishmania*
Lu.	*Lutzomyia*
Ev.	*Evandromyia*
(V.)	*Viannia.*

APRESENTAÇÃO

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SUMÁRIO

Sandflies Naturally Infected by *Leishmania* and Predictors Factors of American Tegumentary Leishmaniasis in Brazil: a Systematic Review

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Abstract

American tegumentary leishmaniasis (ATL) is an anthropozoonosis caused by protozoa of the *Leishmania* genus that affects the skin and/or mucous membranes, and is transmitted to humans from the biting of sandflies. In the Americas, Brazil is responsible for the largest number of cases, and about 51.1% of the Brazilian population lives in areas at risk of ATL transmission. A higher number of epidemiological variants is involved in each region of Brazil, such as the different parasite species, vectors, reservoirs and even hosts. Thus, we conducted a systematic literature review on sandflies naturally infected with *Leishmania* and related predictors in Brazil. We searched for articles in three main databases and did not limit the publication date. Two blinded reviewers selected the studies applying inclusion and exclusion criteria. A third author removed the discrepancies. The data was extracted and the risk of bias was measured by SYRCLE's tool. A total of 7,602 studies were recovered from databases, and after eligibility applying the exclusion and inclusion criteria, 35 studies were included. A total of 206,874 phlebotomine was found to belong to the 17 different genera. The most abundant being *Lutzomyia sp.*, *Nissomyia sp*. and *Psychodopygus sp*, and peridomicile area presented more prevalence. The identification of *Leishmania* occurred mainly through the kDNA-targeted PCR technique. The *Viannia* subgenus was reported by 24 studies, and the species *Leishmania (V.) braziliensis* was the most identified in insects collected, followed by *L. (V.) guyanensis* and *L. (L.) amazonensis.* The Northeast presented the highest index of natural infection in the vectors surveyed, approximately 3.19% (total search, total positive), followed by the Southeast region with about 0.95%, and the North region had an index of 0.76%. A total of 21 studies has a low risk of bias, 12 moderate and two high risks. In Brazil, there is an immense and significant variety of sandflies infected by *Leishmania* in all regions of the country. The capture of sandflies by CDC traps and the identification of natural infection by the kDNA-directed PCR method is one of the most promising techniques to carry out surveillance in ATL endemic areas.

Keywords: Cutaneous Leishmaniasis, vectors, phlebotomine, American Tegumentary Leishmaniasis, Brazil

Background

American tegumentary leishmaniasis (ATL) is an anthropozoonosis caused by protozoa of the *Leishmania* genus that affects the skin and/or mucous membranes. *Leishmania* is transmitted to humans from the biting of sandflies. Brazil is responsible for the largest number of cases in the America region, and about 51.1% of the Brazilian population lives in areas at risk of ATL transmission. Due to the large number of epidemiological variants involved in each region of Brazil, such as the different species of the parasite, vectors, reservoirs, and even hosts, there is some difficulty in collecting significant and robust evidence for the formulation of public policies against the expansion of cases of ATL in the country. We conducted a systematic literature review on sandflies naturally infected with *Leishmania* and predictors of ATL in Brazil.

Introduction

American tegumentary leishmaniasis (ATL) is an anthropozoonosis caused by protozoa of the *Leishmania* genus, presenting great epidemiological diversity and widespread distribution in America [1]. Leishmaniasis is considered a neglected tropical infectious disease and is among the priorities of the World Health Organization (WHO) for the control and elimination of the disease [2]. It is estimated that there are 0.7 to 1.3 million new cases of ATL diagnosed every year worldwide [1], and Brazil is responsible for the largest number of cases in the American region. About 51.1% of the Brazilian population lives in areas at risk of ATL transmission, with males being the most affected due to occupations and leisure activities that increase exposure to these factors, such as agriculture and fishing [3]. Most of the cases were registered in the North, Northeast and Southeast regions of the country [1]. In 2017, the Plan of Action on Leishmaniasis in the Americas was approved, to reduce morbidity and mortality, through the improvement of diagnosis, treatment, surveillance and control of ATL in endemic areas by the year 2022. By understanding the dynamics of ATL transmission and promoting actions of entomological vigilance, it is possible to propose strategies aimed at preventing the disease and reducing the number of new cases [4].

Initially, ATL occasionally affected people in contact with forests, however, changes in the disease transmission pattern have been observed in Brazil. Case reports in rural and peri-urban regions have emerged in recent decades [1]. In areas of geographic expansion, changes in the local ecosystem and changes in the biological behavior of sandflies, as well as the presence of animals, may alter the epidemiology of the disease, assuming characteristics of the home transmission [5,6,7].

ATL is considered a primary zoonosis of wild mammals and secondary of domestic animals, being considered an accidental host. Wild animals are known to act as reservoirs, that is, they ensure the parasite's circulation in nature over a period of time and space. The role of domestic animals in maintaining the parasite in the environment is not definitively clarified. The reservoir-parasite interaction is considered a complex system since it is multifactorial and dynamic [1].

The sandflies play the role of vectors of leishmaniasis, their dynamics are considered of great importance for public health. These insects are distributed over wide areas of the world and are found in abundance in tropical areas, where they can carry out their complete life cycle throughout the year [7].

When the infected female insect bites the vertebrate host, it releases metacyclic promastigote forms in the skin of mammals, which are rapidly phagocytosed by cells of the mononuclear and polymorphonuclear phagocytic system. The parasite adheres mainly to the surface of macrophages passing to the intracellular environment, where it transforms into the amastigote form, characteristic of parasitism in mammals, multiplying until the cell ruptures and are released to infect other macrophages, or infect sandflies, maintaining the parasite cycle and thus spreading the disease [1,8].

The diagnosis of leishmaniasis is based on laboratory and clinical aspects. When the patient has lesions suggestive of leishmaniasis, the presumptive diagnosis can be based on clinical and epidemiological criteria. However, the diagnosis must be confirmed by immunological exams such as Montenegro's intradermal reaction (IDRM) and indirect immunofluorescence, and parasitological exams by direct investigation of the parasite in the lesion, in tissue or biological fluid. The treatment of the first choice is pentavalent antimoniate for intramuscular administration. In Brazil, the most used drug is Glucantime® (pentavalent antimonial), but there are also other therapeutic options such as amphotericin B, since the therapeutic failure, parasitic resistance, adverse effects and toxicity can occur [1].

It is believed that the implementation of bioecological studies of the species identified as vectors is capable of providing useful information to support the elaboration of indicators that may contribute to the assessment of the risk of infection by ATL and, in this way, may generate control measures more effective against the disease [1,7]. Considering the endemicity of ATL in Brazil and other countries of America, the great diversity of vectors and reservoirs, and other epidemiological predictors involved in the transmission of the leishmaniasis that is still unclear, we investigated the epidemiological profile of the ATL in Brazil identifying the invertebrate hosts and predictors factors involved in the transmission of the disease.

Materials and Methods

Study design

A systematic literature review was performed according to the *Preferred Reporting Items for Systematic Reviews and Meta-Analysis checklist* (PRISMA) method [9] (Supplementary file Table S1). Original and comparative articles that analyzed the epidemiological profile of ATL and investigated the reservoirs involved in the transmission of the disease in Brazil were selected for this review. The research question followed the PECOS acronym [10] (population, exposition, comparator, outcome and study design): What are the sandflies naturally infected by *Leishmania* and the predictive factors of tegumentary leishmaniasis in Brazil? (Supplementary file Table S2).

Search strategy

After numerous simulations, the researchers (KLTG, IGD) defined by consensus the most appropriate descriptors and terms for the research. The articles were searched in the Pubmed (U.S. National Library of Medicine), Web of Science (Clarivate Analytics, U.S.), Scopus (Elsevier) and LILACS (Latin American and Caribbean Center on Health Sciences Information) databases. The descriptors (Mesh Terms, Medical Subject Headings) and the terms used were divided into four blocks (Bloco 1: “Leishmaniasis, Cutaneous/epidemiology”; “Leishmaniasis, Cutaneous/transmission”, “*Leishmania*/epidemiology”; Bloco 2: "Disease vectors", "Disease reservoirs", “Psychodidae”, “Dogs”, “Animals Disease”; and Bloco 3: “Brazil”; Bloco 4 (NOT): “Leishmaniasis, Visceral”). The search was done during April 2021 (Supplementary file Table S3).

Inclusion and exclusion criteria

Articles published until 31/12/2021 were included in this review. We included studies on tegumentary leishmaniasis (cutaneous or mucosal or mucocutaneous), infection by *L. (Viannia) braziliensis, L. (Leishmania) amazonensis, L. guyanensis, L. panamensis, L. shawii*, and *L. lainsoni.* As inclusion criteria, only articles that were published in Portuguese or English, had abstract and full text available were selected.

Articles that did not investigate *Leishmania* spp. in a reservoir, studies that had been conducted in another country than Brazil, articles about visceral leishmaniasis, experimental studies, review articles, comments, letters, editorials, theses, dissertations, errata publications, interviews, and guidelines were excluded from this review (Supplementary Table S2). The exclusion criteria were: wrong problem/population (not meeting goals of study); wrong exposition (not investigated *Leishmania* in a reservoir) (e.g., visceral leishmaniasis or *Leishmania* species of Old World, kalazar, post-kalazar, or infection by *L. donovani* and *L. infantum* (or *L.*

chagasi); wrong study (type) (experimental study or vertebrate animal); wrong context (study conducted in another country (not Brazil) (Supplementary Table S2).

Selection of articles

After obtaining the articles through the crossings of the search blocks, the articles were organized in the EndNote software (My EndNote Web, Thomson Reuters) to remove duplicates. For the reading and selection of studies, six researchers belonging to group I (KLTG, THF, KPS, BGS, AAW, KCCS) were randomly distributed in pairs. The first step of article selection was the research for potential publications through the reading of the titles and abstracts, excluding the studies that did not attempt to select criteria. After the first selection, the full articles (reading the PDF) were read and selected independently and blindly, and exchanged between the pairs for review and consensus of the analyzed data. In cases of discrepancies between the pairs, a third reviewer resolved the differences (expert, IGD).

At the final selection, an expert author (IGD) independently assessed the quality of articles included in the study according to the adequacy of publications to the theme of the review, complete information provided by the studies, risk of biases and limitations pointed out by the authors of articles and detected by the reviewers. The articles that did not meet the selection criteria or that had incomplete content were excluded.

Data extraction and analysis of results

The data extraction was performed by structuring the relevant information obtained from the articles in tables format. The research of group I (KLTG, THF, KPS, BGS, AAW), from the selected articles, extracted the data and completed the tables. Then, the experts of group II (EMD, IGD) performed the corrections of the tabulated data, in three steps. The first one consisted of the random distribution of articles among the experts, who promoted the first corrections independently. In the second step, the experts were randomly distributed in pairs and made the review by exchanging the articles with each other. In the third step, the experts made the necessary corrections in pairs.

Risk of bias assessment

We assessed the risk of bias of the individual studies by adaptation of the *checklist* Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE's, NC3RS, Reino Unido) [11]. Two blinded and independent reviewers (AAW, JPF) checked ten items, and the discrepancies were validated by topic expert (IGD). The answers were Yes, No or Unclear. We considered a low risk of bias when 75% reporting 'Yes' for questions, 50-75% moderate and <50% high risk of bias.

Results and Discussion

A total of 7,602 studies were recovered from databases, 2,664 duplicate articles were removed. After removing the duplicity, the exclusion and inclusion criteria were applied and 4,723 articles were removed in the first step due to wrong population (n = 2,119), wrong outcomes (n = 1,365), wrong intervention (n = 802), wrong study design or publication type (n = 436), and full article unavailable (n = 1). In the second stage, 178 articles were excluded due to wrong population (n = 1), wrong intervention (n = 5), wrong outcome (n = 132), wrong study design (n = 19) and wrong publication type (n = 21). 37 articles were selected for the last phase, one study was excluded due to experimental parasitic infection in hamsters and another study was excluded because it focused on visceral leishmaniasis resulting in 35 studies included in this systematic review (Figure 1, flowchart; Supplementary file Table S4).

Studies characteristics

Regarding the type of study selected, all were cross-sectional epidemiological. In twenty-three studies the objective was to evaluate sand fly fauna [12-34]. In eighteen studies, the objective was to evaluate the rate of infection by *Leishmania sp*. in vectors collected [12,16,18,20,25,27,30-41], ten articles evaluated the important epidemiological aspects of the transmission of ATL [15,17,20,22,23,29,32,42,43,46] while the other six to the identification of blood meal sources of insects captured [12,13,16,19,20,38] (Table 1; Supplementary file Table S5).

The first study was published in 1989 in the state of Amazonas and Rondônia [43]. The latest studies were published in 2020 [12,13,14,42]. Seven studies were published in 2017 [21,22,23,24,25,35,44], four studies in 2020 [12,13,14,42] and 2018

[17,18,19,20], three in 2015 [26,27,37], 2013 [29,30,31] and 2010 [32,33,45], two in 2019 [15,16] and one in 2016, 2014, 2012, 2009, 2006, 2004,2002, 1999 and 1998 [28,35,36,38,39,40,41,43,46] (Table 1).

Environment Aspects

The studies covered all regions of Brazil. The majority was performed in the Northern region (eighteen, 51.4%) [12,13,15-20,22,27-29,31,35,36,43-45], following Southeast (seven, 20%) [14,25,32,33,37,38,46] and Northeast (seven, 20%) [21,24,26,39-42], next are South region (two, 5.7%) [23,34] and Central West (one, 2.9%) [30] (Table 1). The Ministry of Health of Brazil related that most of the ATL human cases were registered in the North, Northeast and Central West regions of the country [1]. In 2019, there was a higher prevalence in the number of confirmed cases of ATL in the North region with 7,123 cases, followed by the Northeast region with 3,570 cases, Central-West region with 2,759 cases, Southeast region with 2,444 and South with 239 cases, totaling 16,135 confirmed cases in Brazil [47].

Among the studies included for this review, seven were carried out in the Minas Gerais state [14,25,32,33,37,38,46], five in Acre [12,19,35,36,44] and Pernambuco [21,24,26,39,42], four in Amazonas [18,27,28,43] and Rondônia [13,24,29,43], three in Pará [15,17,45], two in Amapá [20,22], while the states of Paraná [23], Mato Grosso [30], Rio Grande do Sul [34], Bahia [41], Maranhão [40] and Tocantins [31] were explored in an article each (Table 1).

The state of Minas Gerais is the primary target for ATL studies in the Southeast Region, since of the 44,591 confirmed ATL cases in this region, 29,609 were reported in Minas Gerais, in the period 2001-2019 [48]. In the South region, the Paraná state deserves attention since it is responsible for approximately 95% of all reported cases in this region, and stands out with 14,217 reported cases of 14,967 total cases [1]. The analysis of the spatial distribution in Brazil showed a wide dispersion of the disease, but with an intense concentration in some áreas [49], in keeping with the epidemiological reports that record cases of ATL in all regions of Brazil [1] as well as high incidence associated with a transmission index classified as intense in the country [50]. In 2003, all states of Brazil registered autochthonous cases of human leishmaniasis [1].

Considering the location in which the vectors were collected (Table 1), twelve studies reported the peridomiciliary areas as collection site [14,17,19,26,27,33-

36,40,41,46], eleven studies reported the rural areas [18,19,23,24,27,29-31,38,42,44], ten studies reported a forest areas [12,13,17,18,22,24,26,27,34,43], five studies reported urban areas [15,19,29,35,39] and also five reported peri-urban areas [16,20,31,32,33], three domiciliary [34,41,46], three indigenous villages/reserve areas [28,37,42], three conservation unit/Reserve/protection area [16,25,45] and one military training camp area [21] (Table 1).

There was a predominance of the peridomicile areas and rural areas as the location of studies. In urban or peridomicile areas, some studies related to the presence of domestic and wild animals. In the last decades, adaptive and evolutionary processes have been occurring, modifying the epidemiological profile of the disease from rural to urban character [51]. Considering the zoonosis of wild animals that occasionally affected people in contact with forests, the ATL adopted a rural character, currently reaching peri-urban/urban areas [52,53,54].

Considering the diversity of geographical regions, there is a triple epidemiological profile of ATL in Brazil. Endemic cases characterize the first profile in forest areas with the presence of wild animals, as in cases of deforestation in the Amazon region [55]. The second emerges as seasonal outbreaks [49] associated with the increase in extractive activities, agricultural expansion and an opening of roads, allowing an overlap of urban and rural areas. The third profile is related to the migratory process, and to the urban agglomerates linked to residual forests [1] since the frequency and density of sandflies in the peridomicile depend on the presence and distance to these forests [56]. In this last profile, the pattern of occurrence of ATL begins to affect women and children in the domiciliary/peridomiciliary area, with a favorable environment for disease transmission [51]. Also, the presence of domestic animals in the peridomicile and the distance from the shelters of the animals to households can be a determinant of the number of sandflies [56]. This occupation and disordered human invasion in forest areas, allowing the approximation of the vectors to the peridomestic/domicile and the propagation of the disease cycle were also demonstrated with the presence of vectors in wild environments [57].

At the site of a collection of sandflies, an average annual temperature variation was from a minimum recorded of 8°C [23] and a maximum of 33°C [12]. Regarding the altitude (average elevation), the lowest was 115 meters [26], while the highest altitude reported was 2,072 meters [25]. The maximum precipitation related was 2,500 mm [18] (Supplementary file Table S5). Factors such as temperature, relative humidity, and rainfall can influence the population density of sandflies [58], as well as seasons [59,60]. Generally, for the sandflies development, they need temperatures between 20°C and 30ºC, humid areas, low light and close to the ground [61]. Also, they can survive in areas where the average temperature ranged from 16°C to 44°C, and the altitude varied from sea level to 1500 meters [62].

In this review, the study that obtained the capture of a larger quantity of sandflies occurred in an environment of ideal temperature for vector proliferation and one average of 85–95% of humidity [15], demonstrating the importance of environmental conditions in the development of vectors. It is noteworthy, the studies that investigate the seasonality on the sandflies distribution are insurance since they are carried out within at least two years, and thus, to compare the results and to reduce the error bars caused by atypical years of incidence [63].

The catches of the vectors occurred with CDC-type traps in sixteen studies [15,17,18,20-22,24-26,29,30,36,40,42,45,46], in other thirteen were used HP traps [12-14,16,19,27,28,31-33,35,37,38], twelve reported using Shannon type traps [12,15,20,22,23,31-34,41,44,45], two studies used Disney traps [17,43] and one article each used Falcão traps [23] (Supplementary file Table S5).

The insect collection occurred on average for one year [14,15,18,20,23-26,33,35,36,42]. In nine studies, the period lasted two years [16,19,21,29,32,37,44-46] and two for 10 months [27,30]. The longest study was 4 years [43] and the shortest catch period was one month [28]. Three articles did not report the trap used and the period of the capture of the insects [38,39,41] (Supplementary file Table S5). Among the studies, there was a homogeneous distribution of the months/seasons of the year in which the insects were captured. They were captured only in the summer period, catches that started in the summer and ended in spring, as well as those that started in the winter and ended in the fall.

Invertebrates host

The total number of sandflies collected in the 35 studies was 206,874 being found phlebotomines belonging to the seventeen generous: *Brumptomyia, Bichromomyia, Evandromyia, Lutzomyia, Viannamyia, Micropygomia, Migonemyia, Expapillata, Nyssomyia, Pintomyia, Martinsmyia, Pressatia, Psathiromyia, Psychodopygus, Sciopemyia, Trichopygomia*, and *Tricophoromyia*. Of these, 113,567 are females, while 64,106 are males. Four studies did not differentiate the genus of captured sandflies [19,33,39,43]. The genus *Lutzomyia* sp. with 61,201 sandflies was the most collected, followed by *Nissomyia sp*. with 27,489 sandflies and *Psychodopygus sp.* with 10,149 sandflies. These three genera correspond to approximately 48% of all vectors captured. *Lutzomyia sp*. was still the most frequently collected in the studies (85.7% of the total studies), with about 124 different species. The study that presented the highest number of insects collected obtained 25,594 phlebotomines [15], while the minimum obtained was one phlebotomine [39]. It was reported the capture of the sand fly of the genus *Martinsmyia sp*. (n = 1236 insects) [37]. The genus *Expapillata sp*. (n = 2) was found only in one study [23]. Some articles have also reported the capture of the genus *Migonemyia sp*. (n = 944) [15,18,19,20,22,23,27,28,31,35,37,44] (Supplementary file Table S6). There was variation in the density and species of insects collected by region:

Northeast region

The Northeast region presented about 21% (n = 43,957) of all vectors captured in the 35 articles of this review [21,24,26,39,40,41,42]. Five studies of this region occurred in the state of Pernambuco [42,21,24,26,39], one in the state of Maranhão [40] and the other in Bahia [41]. In five studies, just *Lutzomyia sp*. genus was found, and *Lu. whitmani* species was reported in these studies [21,24,26,40,41]. In absolute numbers, the *Lu. choti* was the most abundant (n = 22,270) followed by *Lu. longispina* (n = 6,302) and *Lu. whitmani* (n = 3,627) (Supplementary file Table S6). The abundance *Lu. choti* can be explained by this vector as one of the most common phlebotomine species in remnant forest areas in Brazil [26,64,65], considering that three of the five studies carried out in Pernambuco had collections in forest areas [21,24,26]. Also, this species can feed on humans [66], indicating its performance as a possible ATL vector in these areas.

North region

The North region had the highest number of insects collected (n = 138,269). corresponding to approximately 67% of the total number of insects captured [12,13,15-20,22,27-29,31,35,36,43-45] (Supplementary file Table S6). In this region, there was a predominance of *Nyssomyia antunesi*, *Psychodopygus davisi* and *Psychodopygus paraensis* reported in ten of the eighteen studies [12,15,16,18-20,22,27,28,31,35,44], followed by *Trichophoromyia ubiquitalis* and *Psychodopygus claustrei* cited in nine articles each [15,16,18-20,22,27-29,31,44,45] and *Lutzomyia sherlocki* cited in eight [19,20,22,27-29,35,36,44,45]. The genus with the most collected sand fly was *Nyssomyia sp*. (n = 14,697), followed by *Lutzomyia sp.* (n = 10,588) and *Psychodopygus sp.* (n = 9,727). The largest number of sandflies captured in the eighteen studies was to the *Nyssomyia umbratilis* genus with 6,450 species collected, present in five articles [18,20,22,27,28]. The three states with the greatest diversity of sandflies were Acre with 65 species [36] and Amazonas and Amapá with 54 species each [18,22]. Considering the presence of the Amazon Rainforest in this region, the possibility of finding a restricted range of different sand fly species is high in these states [67]. Making this region a critical area for the transmission of ALTs in the current decade, especially since 2010, contributing with approximately 59% (n = 122,141) of total the phlebotomines collected in 35 studies, the highest among all regions. There is a close relationship between regional and local migratory movements, such as migrations to the south of the Amazon generated by the construction of roads in the 1960s and 1970s, gold mining between 1970 and 1980 and extraction between 1980 and 1990 with the emergence of several outbreaks epidemic and generally associated with the most vulnerable populations in areas of environmental and social dynamics [51].

Southeast region

The third region that most reported vector capture was the Southeast, with approximately 13% of all vectors (n = 27,462) [14,25,32,33,37,38,46]. There was a prevalence of the vector *Lu. longipalpis* reported in five of the seven studies in this region [14,25,33,37,46], followed by *Nyssomyia whitmani* and *Evandromyia termitophila* reported in four studies [14,25,33,37] and *Psychodopygus lloydi, Evandromyia lenti, Lutzomyia ischnacantha* and *Nyssomyia intermedia* all reported in three studies [14,25,33,37,38]. In quantitative terms, *Nyssomyia whitmani* was the most abundant species (n = 12,082), followed by species *Lu. longipalpis* (n = 5,559) and *Micropygomyia migonei* (n = 1,533) [14,25,33,37,46] (Supplementary file Table S6).

Nyssomyia whitmani was the predominant species in the study. This species has been frequently found in endemic areas for ATL in the Northeast and Southeast regions of Brazil, being incriminated as one of the main vector species of *L. braziliensis* in some regions of the country, moreover, has demonstrated a considerable degree of synanthropic and adaptation to the urban environment [68] and is considered a highly anthropophilic species [69]. *Lu. longipalpis* is considered the main vector species of *L. infantum* in Brazil [70]. These sandflies are characterized by sheltering in primary and secondary forests, and limestone rocks, preferably [71]. In Brazil, the species *Lutzomyia longipalpis*, can adapt to different environments, including urban and peri-urban environments with a high degree of synanthropic [70]. *Migonemyia migonei* was the third most captured species, and has great health importance as it is considered an anthropophilic species [72]. *Martinsmyia minasensis* is the fourth most abundant species of sandflies (n = 1,236) [37], whose feeding habits may be closely related to rodents in the study area [73].

Central West region

The Central West region comprised approximately 2% of all vectors captured (n = 3,743) [30]. About the amount collected, the species *Lutzomyia antunesi* was the most abundant (n = 1,701), followed by *Lutzomyia saulensis* (n = 770) and *Lutzomyia walkeri* (n = 494) (Supplementary file Table S6). With the expansion of agriculture and livestock frontiers, in which the region became an important route for the establishment of man, the epidemiological pattern of transmission of ATL was modified to urban centers, reaching less favored populations and increasing the numbers of cases, especially from the first decade of the twentieth century [51].

Southern region

Finally, the southern region represented 2% of all vectors identified (n = 3,443) [23,34] Studies in this region were carried out in Paraná [23] and Rio Grande do Sul [34], states with the highest number of ATL cases in the southern region (Supplementary file Table S6). The most collected and identified species was *Lutzomyia migonei* (n = 1,134), followed by *Brumptomyia brumpti* (n = 607) and *Pintomyia fischeri* (n = 440). Among the species captured in the territory of Paraná, the one that prevails is *Brumptomyia brumpti*, and none of the 52 vectors analyzed presented infection by Leishmania. This genus is widely found in animals, notably the armadillo, indicating a possible association between these phlebotomines and the armadillo, known as *Leishmania* host [74].

In the state of Rio Grande do Sul, the predominant genus was *Lutzomyia sp.* (n = 2,220). *Leishmania (V.)* infections were found by DNA amplification of two *Lutzomyia pessoai* and one *Lu. missionensis*. with the first report of natural infection with *Leishmania (V.) sp*. in *Lu. personali,* it is considered a likely vector in other endemic areas of Brazil. *Lu. misionensis* are found both in the domicile environment and in the forest. It is endowed with a remarkable degree of anthropophilic, and its role in the transmission of *Leishmania braziliensis* and secondary in the Southeast and Northeast regions of Brazil [72].

***Leishmania* natural infection *versus* invertebrate host**

An essential step for the incrimination of *Leishmania* vectors is the occurrence of naturally infected sandflies [75]. Thus, we also search for articles that investigated the naturally infected vectors. In 33 studies DNA samples from female sandflies were obtained to detect *Leishmania* infection [12-42,44,46]. One study examined only *Leishmania* infection through a hamster insect sample [45] and one study did not report the method for detecting Leishmania naturally infection [43] (Supplementary File Table S7).

In this review, the identification of *Leishmania* in the vector occurring by conventional polymerase chain reaction (PCR) was the method of choice for fifteen studies [13,14,16,17,19,24,26,28,29,33,34,40,41,44,46]. The restriction fragment polymorphism analysis using the polymerase chain reaction (PCR-RFLP) occurred in eleven studies [15,20,22,25-,27,32,33,36-39]. Shoot sequencing [12,27,30,33,35,37] and the multiplex PCR [12,18,23,31,35,36] was performed for detection in six studies each, follow by bot dot hybridization in three studies [31,35,41] and Leishmania-specific nested polymerase chain reaction (LnPCR) in two studies [30,37]. Multilocus enzyme electrophoresis (MLEE) [39], real-time PCR, enzyme restriction analysis [21], specific monoclonal antibodies [45], indirect immunofluorescence method (MCAb), Isoenzyme electrophoresis [22] and Fast multiplex Real-time PCR [42] were methods of choice in one article each (Table 2; Supplementary File Table S7). The use of molecular techniques, such as PCR, increased the sensitivity and specificity of parasite identification [76]. The efficacy of this methodology can explain studies using the PCR and PCR-RFLP technique in detecting the parasite independent of the infection stage and whether or not it is located in the digestive tract of the vector. Moreover, its practicality and simplicity, provide identification with high sensitivity [77]. However, it is necessary and important to verify the standardization of the methods used during the storage and extraction of samples [78].

Of those who chose to perform the PCR technique, the most commonly used target was the kDNA region, used in seventeen studies [12,13,16,18,21,23,26,27,29,31-36,41]. The region hsp70 was explored in seven studies [12,13,16,27,35,36,38], followed by the ITS1 region in six articles [14,19,25,28,37,38], the RNA polymerase II gene in two studies [20,22]. The other regions, such as SSUr DNA [37], SSUr RNA [30], ITS rDNA [39], IVS6 [35], SL RNA [36], TspRI, HgaI endonuclease [15] and Mini-exon gene [17] were targets of the technique in one work each. Six studies not related to the PCR target [24,40,43-46] (Table 2; Supplementary File Table S7). To date, several molecular targets have been reported for the search for Leishmania genetic material in PCR assays. However, kDNA stands out as the most studied target due to its high number of copies per genome of the parasite and the distinct presence of kinetoplast in trypanosomatids, which produce high sensitivity and detection of the parasite in clinical samples in different forms of leishmaniasis [79-82]. The hsp70 gene region is widely used in PCR-RFLP since it allows the diagnosis of ATL with the capacity to differentiate the disease-causing species in Brazil [83]. ITS regions are used for species typing, reflecting high variability between these species [84,85]. According to the specification of PCR combined with the use of primers that amplify specific sequences of the parasite, the studies of vector incrimination are potentiated [78].

Summarizing Table 2, of the 53,580 vectors of 35 articles that obtained DNA samples from female sandflies, 367 samples plus 134 pools of samples were positive for the genetic material of the parasite. Five studies did not detect the genetic material of the parasite in samples obtained [23,24,26,29,46]. Evaluating by region, the Southeast presented the study with the highest index of natural infection in the vectors surveyed, approximately 39.62% (total search, total positive) [32]. Another region that obtained the highest index was the North, with studies with 27.08%, 14.73% and 13.79% of natural infection [20,27,31]. In the northeast region, the study with the highest rate was 12.4% [42] while the Center-West region had the highest index of 4,43% [30] and the southern region had the highest index of 0.32% [34]. These proportions of natural infection of vectors suggest that variations in *Leishmania* transmission at each site will probably also depend on the behavior and vector competence of the predominant species, rather than just differences in *Leishmania* prevalence [86].

The *Viannia* subgenus was reported by 24 studies, and the species *Leishmania (V.) braziliensis* was the most identified in insects collected, and it was reported in 19 studies [12-14,16,19,21,25,28,30-33,35-39,45,46]. The parasite was identified in 88/14,246 samples and 23/2,840 sand fly pools. *Leishmania (V.) guyanensis* was reported in seven studies and was identified in 48/7,330 samples and 27/1,950 sand fly pools [12,19,20,22,30,36,37]. *Leishmania (L.) amazonensis* was reported in five studies and was identified in 2/2,432 samples and 10/1,682 sand fly pools [14,17,28,37,43]. *Leishmania (V.)* spp. was reported in three studies and was identified in 3/920 samples and 30/1371 sand fly pools [28,34,37]. The *Leishmania infantum chagasi* (visceral leishmaniasis (VL) agent) was reported in one study and was identified in turn in 7/1,289 sand fly pools [37]. *Leishmania (V.) lainsoi* was reported in two studies and was identified in 1/1,881 samples and 7/95 sand fly pools [15,27]. One study reported *Leishmania (V.) shawi* in only 1/95 sand fly pools [27]. *Leishmania (V.) naiff* was reported in two studies and identified 3/6,212 samples and 1/194 sand fly pool [13,22] (Table 2; Supplementary file Table S7).

The high frequency of *Leishmania braziliensis* is justified by this species present widely distributed throughout Brazil, occurring in all region. It is worth mentioning that the ecoepidemiology of ALT as a result of *Leishmania (V.) braziliensis*, as well as some of their vectors characteristics in each Brazilian region [86]. The transmission of *Leishmania (V.) braziliensis*, in altered environments, occurs in domiciliary spaces, affecting any age group and individuals of both sexes. The lesions are resulting from the infection present as typical single or multiple skin ulcers. The principal complication is hematogenic dissemination and involvement of the mucous membranes, especially the nasopharyngeal mucosa. The treatment must take into account the infecting species, evaluating the geographic region and the eco-epidemiological profile of the vectors and reservoirs present at the site. The response to the specific treatment recommended presents variable results. Some individuals require complementary therapies to obtain the clinical cure [1].

In some studies, not all infected phlebotomine were able to characterize the species of the parasite, probably due to the small amount of vector DNA, generating low-quality techniques [13,14,23,26,35].

Northern Region

The studies of the Northern Region, especially those in Acre and Amazonas, showed a higher prevalence of the natural infection in the phlebotomines studied by the species *Leishmania braziliensis*, a species proven to be an ATL agent [87,88]. This agent was found in twenty distinct species of sandflies, with high frequency in *Psychodopygus davisi*.

Interestingly, in addition to the detection of *Leishmania braziliensis* DNA in *Ps. davis*, it was also found in *Ev. saulensis* and *Pressatia sp*., corresponding to the first record of possible infection by *Leishmania (V.) braziliensis* associated with these sandflies [23]. For the genus *Evandromyia*, there are reports of other species, such as *Ev. cortelezi* and *Ev.* saullesi, naturally infected with *Leishmania infantum* in Minas Gerais [89,90]. Findings of natural infection by *Leishmania (V.) lainsoni* and *Leishmania (V.) shawi* in species of *Trichophoromyia ubiquitalis* also indicates a possible association of this vector with the ATL transmission cycle in the Amazon Region. However, some studies have pointed to the presence of the parasite species found in this review [91-93]. The data here pointed out to identify a great variety of vectors and agents with potential for involvement in the ATL transmission cycle in this region.

Northeast region

In the Northeast region, five studies being carried out in Pernambuco [21,24,26,39,42] and only *Leishmania braziliensis* species were identified in the sandflies collected. *Lutzomyia whitmani* with 1/1 sample and 30/1,003 *Lutzomyia choti* with *Leishmania (V.) braziliensis* were reported positive for the infection [21,39]. It is suggested that the vector *Lutzomyia. whitmani* may be involved in the transmission of the wild (enzootic) and peridomestic (zoonotic) cycles of the disease [94]. Highlight *Lutzomyia whitmani* as the main vector of ATL for humans, particularly in peridomiciliary areas, such as near animal shelters and crop plantations. Areas of forest remnants, such as the Atlantic Forest of Pernambuco, are often the capture of *Lutzomyia Whitmani* [64,65]. About *Lutzomyia choti* identified with the parasite DNA, a study carried out in a forest area in Pernambuco, several copies of *Lutzomyia choti* were attracted to the human baits [66], suggesting the attraction of this phlebotomine by man. In addition to the vectors already mentioned, *Leishmania (V.) braziliensis*, in the state of Pernambuco, was previously isolated and characterized in another species of the genus *Lutzomyia*, as *Lutzomyia migonei* [1].

Southeast region

In the Southeast region, there was also a higher frequency of infection by the *Leishmania braziliensis* species [14,25,32,33,37,38]. The phlebotomine already demonstrated vectors of ATL in this region: *Lutzomyia whitmani* and *Lutzomyia neivai*. *Psychodopygus lloydi* was reported infected by *Leishmania (V.) braziliensis* [14,25,38]. A lower frequency and rate of infection were reported to other species of the genus *Lutzomia*: *Lu. christenseni*, *Lu. personally*, *Lu. aragaoi*, *Lu. fischeri*, *Lu. lenti*, *Lu. lutziana* and *Lu. Monticule* [32,37]. *Leishmania braziliensis* was confirmed in one Complexo cortelezzii [33]. There are indications that *Lu. fischeri* presented the potential and ability to transmit *Leishmania braziliensis* in the wild cycle. Ratifying this, it was reported the presence of genetic material of the parasite in this sand fly [95]. Already *Lu. lutziana* has also been reported for the first time infected by *Leishmania (V.) braziliensis* requiring further studies to assess its true potential in ATL transmission [32]. Species of sandflies collected and presenting natural infection, such as *Mastinsmyia minasensis,* still present their role as an ATL vector that has not yet been fully elucidated [35].

Central-West region

In the Central-West region, it was possible to identify natural infection in *Lutzomyia antunesi* by *L. (V.) brazilienses*, *L. (V.) guyanensis* and *L. infantum chagasi*. Also was detected *L. (V.) braziliensis* and *L. (V.) guyanensis* in *Lu*. *ubiquitalis* [30]. In Mato Grosso, the primary vector species of ATL is *Lu. whitmani* [96].

One study detected *Leishmania infantum chagasi* in *Ev. Lenti* [37], and *Evandromyia* sandflies were associated with cases of ATL in Mato Grosso [97], Mato Grosso do Sul [59] and Goiás [98]. In these studies, *Leishmania* detection was done using molecular techniques.

Also, in the *Lu. antunesi* was found in the presence of *L. infantum chagasi* [30], suggesting the possible association of this vector in the transmission of VL in ATL areas. Other studies have also reported vectors that have already been associated with infected ATL transmission *L. infantum chagasi*, indicating a possible co-infection in the vector. A species of *Lu.intermedia*, *Lu. neivai* and *Lu. whitmani* (ATL vectors) in endemic regions of VL, in which the *Lu. longipalpis* (classical VL vector) was absent [99].

South region

In the southern region, we found two studies in two states [23,34]. In the state of Rio Grande do Sul, *L. (Viannia)* was found in two *Lu. personal* and one *Lu. misionensis* for a total of 920 samples analyzed by PCR [34]. In the state of Paraná, no natural infection by *Leishmania* sp. was detected in the collected sandflies [23]. In contrast, a study conducted in Paraná State reported *Lu. whitmani* females infected by *L. (Viannia) sp*. detected by multiplex PCR, although no female was positive to flagellates by dissection [100]. Another study also reported a natural infection in sandflies by the dissection method, and an infected *Nyssomyia whitmani* female was detected [101]. Although no natural infection has been reported in Paraná state, the vectorial captivity of the insects should not be neglected, since the ATL is endemic in the South region.

Risk of bias

The risk of bias of studies applying the adapted SYRCLE’s tool (figure 2) showed 2/35 studies with high risk of bias (5,7%) [21,43], 12/35 moderate (34,3%) [15,24,30,31,34,36,38-,41,44,45] and 21/35 low risk (60%) [12-14,16-20,22,23,25-29,32,33,35,37,42,46].

Some studies presented biases and limitations. In three studies it was reported that a low number of sandflies analyzed may have reduced the probability of finding a positive female [16,24,26]. Five articles reported that it was not possible to determine the species of *Leishmania*, this fact occurred because the DNA fragment provided insufficient sequencing results [13,14,23,26,35]. Due to heavy rains in two studies, the collection of insects was impaired [21,44]. In one study they were unable to identify 26 sandflies collected due to the loss of morphological structures useful in taxonomic classification, and another 119 were classified only in their general, for the same reasons [28]. The studies also point out the need to carry out more studies with a more extensive and more periodic sampling so that a stronger association between the vector competition, the parasite species and the eco epidemiological factors in the transmission of ATL in a given region occurs (Supplementary file Table S8).

Strengths and limitations

In this systematic review, all steps were performed in pairs and validated by an expert. No time was limited to literature search, and this is the first time that the risk of bias was applied to sandflies studies. Some limitations were considered regarding an expressive heterogeneity between studies, such as sandflies capture, *Leishmania* detection methods and outcomes measurements. Because of these factors, a meta-analysis was not conducted. Also, we believe that to expand the search for studies that investigate the epidemiological vectors and predictors of visceral leishmaniasis in Brazil and its interrelation with ATL in different aspects. We also did not apply the GRADE recommendations in this review on sandflies, since there is no consensus to use it between authors and present literature.

Conclusion

In Brazil, there is an immense and significant variety of sandflies infected by *Leishmania* in all regions of the country, from the North to the South of the country, mainly in peridomicile areas. In this study, 17 different genera were captured, of which *Lutzomyia* sp. was the most collected in the studies, with about 124 different species. In the northeast region, the sand fly *Lutzomyia choti* was the most captured, in the northern region it was N*yssomyia umbratilis*, in the southeast region we had the prevalence of *Nyssomyia whitmani*, in the central west region of *Lutzomyia antunesi* and finally in the southern region of *Lutzomyia migonei*. The southeast presented the study with the highest index of natural infection in the vectors surveyed, and *Leishmania (V.) braziliensis* was the most identified species. Therefore, it is crucial to monitor and maintain these actions in research into infestation by sandflies in endemic areas for ATL for the control of infection in humans and other animals. Given the methodologies studied by the articles included in this review, the capture of sandflies by CDC-traps and the identification of natural infection by the kDNA-directed PCR method is one of the most promising techniques for this surveillance.

Abbreviations

ATL: American tegumentary leishmaniasis; WHO: World Health Organization; IDRM: Montenegro's intradermal reaction; PRISMA: Systematic Reviews and Meta-Analysis checklist; PECOS: Population, exposition, comparator, outcome and study design; VL: Visceral leishmaniasis; DNA: desoxyribonucleic acid; RNA: Ribonucleic acid; PCR: Polymerase Chain Reaction; RFLP: restriction fragment length polymorphism; LnPCR: Leishmania-specific nested polymerase chain reaction; MLEE: Multilocus enzyme electrophoresis; MCAb: Indirect immunofluorescence method; KDNA: Kinetoplast DNA; hsp70: 70 kilodalton heat shock proteins; ITS1: Internal transcribed spacer 1 region; SSUr DNA: Small subunit ribosomal DNA; SSUr RNA: Small subunit ribosomal ribonucleic acid; HgaI: Haemophilus gallinarum; SL RNA: Spliced Leader RNA; ITS rDNA: rDNA Transcript Internal Space; *L.: Leishmania; Lu.: Lutzomyia; Ev.: Evandromyia; (V.): Viannia.*

Declarations

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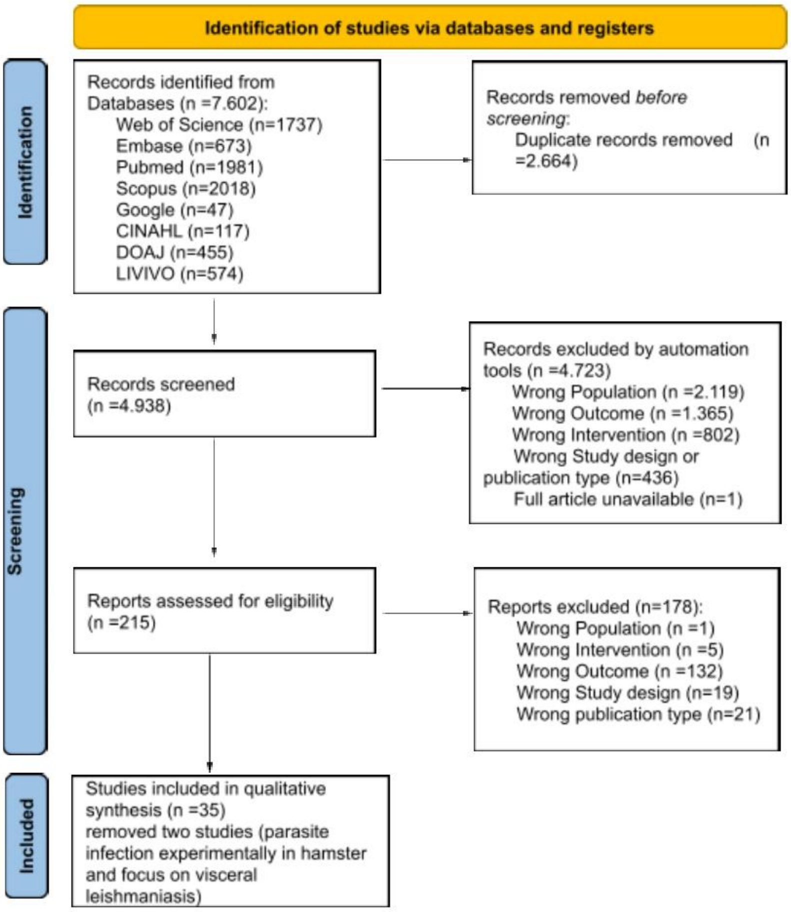
Conflict of Interest: The authors declare no conflict of interest

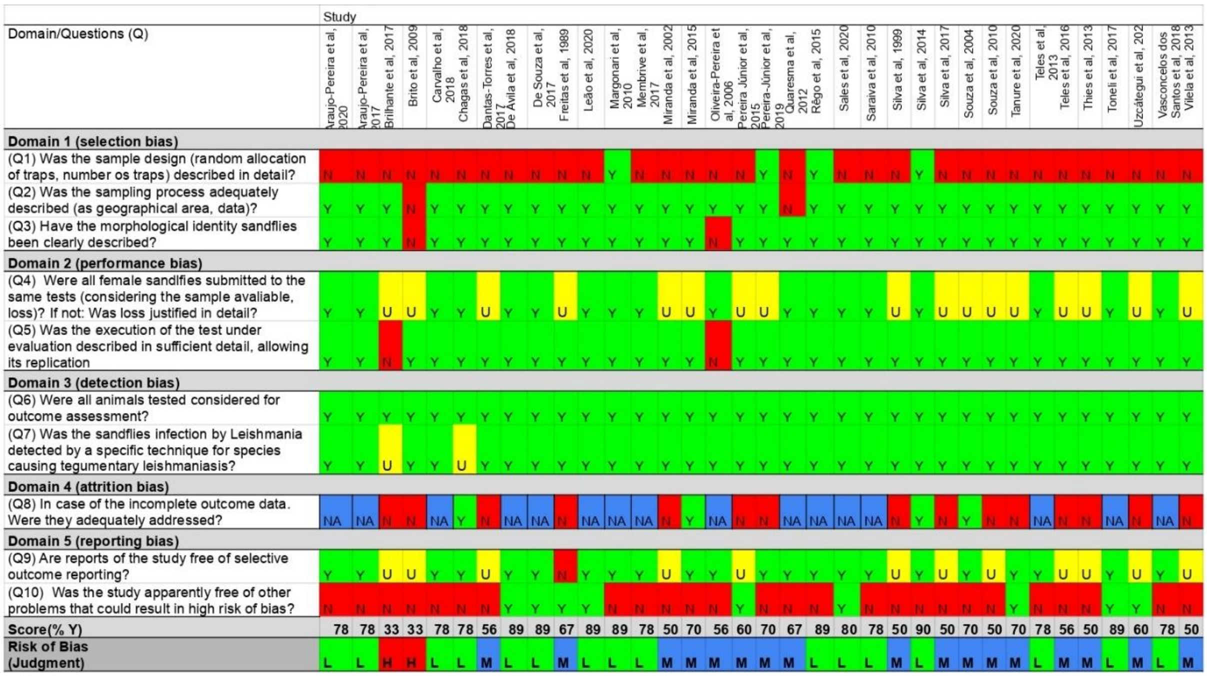
Tables and figure (legend)

Fig. 1 PRISMA 2020 flow diagram for new systematic reviews which included searches of databases and registers.



From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

Fig. 2 SYRCLE's tool for assessing risk of bias (N: No; Y: Yes; U: Unclear; NA: Not applied. L: Low risk of bias (>75%); M: Moderate risk of bias (50-74%); H: High risk of bias (<50%).



Domain/Questions (Q)	Araujo-Pereira et al, 2020	Araujo-Pereira et al, 2017	Brilhante et al, 2017	Brito et al, 2009	Carvalho et al, 2018	Chagas et al, 2018	Dantas-Torres et al, 2017	De Ávila et al, 2018	De Souza et al, 2017	Freitas et al, 1989	Leão et al, 2020	Margonari et al, 2010	Membrive et al, 2017	Miranda et al, 2002	Miranda et al, 2015	Oliveira-Pereira et al, 2006	Pereira Júnior et al, 2015	Pereira-Júnior et al, 2019
Domain 1 (selection bias)																		
(Q1) Was the sample design (random allocation of traps, number os traps) described in detail?	N	N	N	N	N	N	N	N	N	N	N	Y	N	N	N	N	N	Y
(Q2) Was the sampling process adequately described (as geographical area, data)?	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
(Q3) Have the morphological identity sandflies been clearly described?	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y
Domain 2 (performance bias)																		
(Q4) Were all female sandlfies submitted to the same tests (considering the sample avaliable, loss)? If not: Was loss justified in detail?	Y	Y	U	U	Y	Y	U	Y	Y	U	Y	Y	Y	U	U	Y	U	U
(Q5) Was the execution of the test under evaluation described in sufficient detail, allowing its replication	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y
Domain 3 (detection bias)																		
(Q6) Were all animals tested considered for outcome assessment?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
(Q7) Was the sandflies infection by Leishmania detected by a specific technique for species causing tegumentary leishmaniasis?	Y	Y	U	Y	Y	U	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Domain 4 (attrition bias)																		
(Q8) In case of the incomplete outcome data. Were they adequately addressed?	NA	NA	N	N	NA	Y	N	NA	NA	N	NA	NA	NA	N	Y	NA	N	N
Domain 5 (reporting bias)																		
(Q9) Are reports of the study free of selective outcome reporting?	Y	Y	U	U	Y	Y	U	Y	Y	N	Y	Y	Y	U	Y	Y	U	Y
(Q10) Was the study apparently free of other problems that could result in high risk of bias?	N	N	N	N	N	N	N	Y	Y	Y	Y	N	N	N	N	N	Y	N
Score(% Y)	78	78	33	33	78	78	56	89	89	67	89	89	78	50	70	56	60	70
Risk of Bias (Judgment)	L	L	H	H	L	L	M	L	L	M	L	L	L	M	M	M	M	M

Domain/Questions (Q)	Quaresma et al, 2012	Rêgo et al, 2015	Sales et al, 2020	Saraiva et al, 2010	Silva et al, 1999	Silva et al, 2014	Silva et al, 2017	Souza et al, 2004	Souza et al, 2010	Tanure et al, 2020	Teles et al, 2013	Teles et al, 2016	Thies et al, 2013	Toneli et al, 2017	Uzcátegui et al, 202	Vasconcelos dos Santos et al, 2018	Vilela et al, 2013
Domain 1 (selection bias)																	
(Q1) Was the sample design (random allocation of traps, number os traps) described in detail?	N	Y	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	N
(Q2) Was the sampling process adequately described (as geographical area, data)?	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
(Q3) Have the morphological identity sandflies been clearly described?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Domain 2 (performance bias)																	
(Q4) Were all female sandlfies submitted to the same tests (considering the sample avaliable, loss)? If not: Was loss justified in detail?	Y	Y	Y	Y	U	Y	U	U	U	U	Y	U	U	Y	U	Y	U
(Q5) Was the execution of the test under evaluation described in sufficient detail, allowing its replication	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Domain 3 (detection bias)																	
(Q6) Were all animals tested considered for outcome assessment?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
(Q7) Was the sandflies infection by Leishmania detected by a specific technique for species causing tegumentary leishmaniasis?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Domain 4 (attrition bias)																	
(Q8) In case of the incomplete outcome data. Were they adequately addressed?	NA	NA	NA	NA	N	Y	N	Y	N	N	NA	N	N	NA	N	NA	N
Domain 5 (reporting bias)																	
(Q9) Are reports of the study free of selective outcome reporting?	Y	Y	Y	Y	U	Y	U	Y	U	Y	Y	U	U	Y	U	Y	U
(Q10) Was the study apparently free of other problems that could result in high risk of bias?	N	N	Y	N	N	N	N	N	N	Y	N	N	N	Y	Y	N	N
Score(% Y)	67	89	80	78	50	90	50	70	50	70	78	56	50	89	60	78	50
Risk of Bias (Judgment)	M	L	L	L	M	L	M	M	M	M	L	M	M	L	M	L	M

Source: Own elaboration.

Table 1 Summary of the characteristics of included studies (n =35). (T= average annual of temperature; R= annual Rainfall; A= Altitude (average elevation); NR= Not reported; GC= geographical coordinates; U= Urban; PU= Peri-urban; P= Peridomiciliary; R= Rural; AP= Another place; Y= Yes; N= No. A= Animal or V= Vector; Ad= Animal (domestic); Aw= Animal (wild); An= Animal (neighborhood); F= Female; M= Male; NR= Not reported; SISA= To evaluate species abundance the index of species abundance "ISA" and the standardized index of species abundance "SISA" were calculated).

Author, year/ State of Brazil	**Objective**	**Location**	**Number and gender of specimens collected**
Araujo-Pereira et al, 2020 / Acre	Data concerning the sandfly fauna of Brasiléia municipality, *Leishmania* DNA-detection rates and the identification of blood meal sources of insects captured in 2013-2015 are presented.	P; AP = high-density forest	61 species and 15 genera / 4,473 (F = 2,297; M = 2,176)
Leão et al, 2020 / Rondônia	To evaluate sand fly fauna from two vertical stratification layers in order to identify potential vectors and their blood-meal sources.	AP =Forest	68 species / 15,457 (Potential vectors identified 10,197 = F = 5,656; M= 4,541)
Sales et al, 2020 / Pernambuco	The general objective was to gather epidemiological data that could indicate the occurrence of a peridomestic/domestic transmission cycle of L. braziliensis in indigenous villages.	R;AP = indigenous villages	5,640 (F = 2,100; M = 3,540)
Tanure et al, 2020 / Minas Gerais	To describe the sand fly fauna and detect trypanosomatids in these insects from Casa Branca, state of Minas Gerais, Brazil, an endemic area of both visceral (VL) and tegumentary leishmaniasis (TL).	P	23 species and 8 gene /16,771 (F= 6,612; M=10,159)
Uzcátegui et al, 2020 / Pará	To investigate, in an urban park of Belém, the phlebotomine sand fly fauna, associated or not associated with *Leishmania* spp. infections, spatiotemporal fluctuation patterns (monthly frequency and vertical stratification), and potential	U = (forested sites)	19 species/ 25,594 (F= 15,705; M= 9,889)

	implications of these findings in the transmission of ACL agents.		
Pereira-Júnior et al, 2019 / Rondônia	To characterise the sandfly fauna and identify their blood meal sources, as well as to assess the natural infection caused by *Leishmania* in RO.	PU; Ap=Forest Edge and Conservation Unit (CUN, forest).	73 species and 14 genera / 9,535 (F= 4,089; M= 5,446)
Carvalho et al, 2018 / Pará	To investigate the ecoepidemiology of ATL in municipalities within the Bragança region of Pará State where human infections of *L. amazonensis* have been recorded recently. These locations were selected with the aim of assessing how the sampling of *Lu. flaviscutellata* can be used to optimize eco-epidemiological surveys of CL in transmission areas of *L. amazonensis*.	P; Ap = Forest	1,394 (F=1,190; M= 204)
Chagas et al, 2018 / Amazonas	To describe the composition and distribution of sand fly species diversity among ecotopes (intradomicile, peridomicile and forest) in an area of ACL transmission, as well as to detect natural infection with *Leishmania* DNA, in order to evaluate which vectors are inside houses and whether they represent a hazard of transmission.	R; Ap= Forest	2,469 (F= 1,759, M= 710
De Ávila et al, 2018 / Acre	To investigate the sand fly fauna of rural and urban environments, and determine their food source and natural infection by *Leishmania* in an endemic area of ATL.	R, U, P	2,517 (NR)
Vasconcelos dos Santos et al, 2018 / Amapá	To assessed potential ATL transmission cycles in the lower Oyapock River Basin to promote knowledge on phlebotomine ecology, mainly focusing on species composition, multi-trapping stratification, blood-source investigation and natural *Leishmania* spp. infections.	PU	9,119 (F= 5,073; M= 4,046)
Araujo-Pereira et al, 2017 / Acre	To evaluate the rates of infection by *Leishmania* spp. in non-blood-fed female sandflies captured in Rio Branco municipality.	U , P	456 (F=256; M=200)
Brilhante et al, 2017 / Acre	To verify the phlebotomine species and traps attractiveness to them in the Amazonian forest of the Acre basin, using white and black Shannon traps.	R	6,309 (F= 5,445; M= 864)
Dantas-Torres et al, 2017 / Pernambuco	To investigate the population dynamics of sandflies in a military training camp located in a remnant of Atlantic rainforest in northeastern Brazil.	Ap = Military training camp	24,606 (F=11,923; M=12,683)
De Souza et al, 2017 / Amapá	The aim of the present survey of the Serra do Navio phlebotomine population is in part to fill this gap by assessing putative transmission cycles in this Brazilian region of the Guiana Shield.	Ap = Primary forest	8,685 (F= 6,212; M= 2,473)
Membrive et al, 2017 / Paraná	To better understand the dynamics of *Leishmania*, sand flies and reservoirs in an anthropic environment in an endemic area of CL.	R	1,215 (F=625;M=590)
Silva et al, 2017 / Pernambuco	To contribute knowledge of the phlebotomine fauna in an area endemic for ACL in northeastern coast of Brazil.	R, Ap= Forest	2,174 (F=1,267; M=907)
Toneli et al, 2017/ Minas Gerais	To describe the patterns of species richness and diversity of sandflies among areas of Caraça Sanctuary and to investigate their seasonal variation. It also aims to assess the presence of *Leishmania* DNA among these insects.	Ap = Reserve (Santuário da Caraça)	376 (F= 300; M= 76)
Teles et al, 2016 / Acre	To identify the phlebotomine sandfly vectors involved in the transmission of ACL in Assis, Brazil.	P (rural areas roads)	6,850 (F= 3,370;M= 3,480)
Miranda et al, 2015 / Pernambuco	To assess the ecology of sand flies, including *Lu. whitmani*, in a low-density residential rural area with mixed forest/agricultural exploitation in north eastern Brazil	Ap = Forest, P (residential rural with mixed forest/agricultural)	5,167 (F= 2,419;M= 2,748)

Pereira Júnior et al, 2015 / Amazonas	To study and compare the abundance and diversity of sandfly fauna in varzea and terra firme environments, and to detect *Leishmania* DNA in sand flies in an area of endemic ACL.	R, P, Ap = Forest	5,716 (F = 2,848;M= 2,868)
Rêgo et al, 2015 / Minas Gerais	To detect the survey for *Leishmania* DNA among phlebotomine sand flies collected in a village located in the XIR where autochthonous cases of ACL have been reported since 2001.	Ap = Indigenous reserve	4,760 (F=4,760;M=0)
Silva et al, 2014 / Amazonas	To study the sand fly fauna collected during an 8-d surveillance of different habitats	Ap = indigenous reserve	1,267 (F= 819;M= 448)
Teles et al, 2013 / Rondônia	To identify the species of phlebotomine sandflies that may have been transmitting the ACL, and describe epidemiological aspects of disease.	U, R	1,935 (F= 1,240; M= 695)
Thies et al, 2013 / Mato Grosso	To describe the local phlebotomine sandfly fauna and to investigate the infection rates and infecting *Leishmania* species in the captured sandflies.	R	3,743 (F= 2,735;M= 1,008)
Vilela et al, 2013 / Tocantins	To contribute to the current knowledge of phlebotomine fauna in Tocantins and to identify putative ACL vectors in a rural settlement area and in the periurban environment of Guaraí.	R, PU	3,530 (F= 1,658; M=1,872)
Quaresma et al, 2012 / Minas Gerais	To evaluate bioecological features associated with female sandfly food sources and the detection of *Leishmania* sp. in sandflies collected at Ibitipoca State Park, in Minas Gerais.	R: At a small farm located at the boundary of the state park	38 (F=38;M=0)
Margonari et al, 2010 / Minas Gerais	To better understand the epidemiology of leishmaniasis in Divinopolis, the authors evaluated the phlebotomine sandfly fauna and associated to *Leishmania* infections in the Gafanhoto Park.	Ap = Forest remnant located in the PU area (preserved area and in an in an altered area with introduced vegetation)	824 (F=342;M=482)
Saraiva et al, 2010 / Minas Gerais	To detect *Leishmania* infection in the phlebotomine sand flies collected in the northeast sanitary district (NSD) by dissection and molecular approaches.	P; PU	243 (NR)
Souza et al, 2010 / Pará	To evaluate the phlebotomine fauna in the area and its possible role in the transmission of agents of ACL.	Ap = Environmental protection area	22,095 (F= 15,306; M= 6,789)
Brito et al, 2009 / Pernambuco	To identify the *Leishmania* species circulating in the Atlantic rainforest (Zona da Mata) in the Pernambuco state to better understand the observed genetic polymorphisms in previous studies.	U	1 (NR)
Oliveira-Pereira et al, 2006 / Maranhão	To investigate the natural infection rate of three species of phlebotomines in the peridomestic environment.	P	1,100 (F= 1,100)
Souza et al, 2004 / Minas Gerais	To provide data for optimization of control measures, upon knowing seasonal fluctuations,habitat and the behavior of phlebotomine sand flies.	P; Ap= Domiciliary	4,450 (F=1,306; M=3,144)
Miranda et al, 2002 / Bahia	They have tested the hypothesis that there is a clustering of infected vectors by combining a spatial stratification of sample harvesting and analysis in pools of vectors with a very sensitive *L. braziliensis* kDNA minicircle specific PCR and a dot blot hybridization procedure in different sectors of the Corte de Pedra area.	P; Ap= Domiciliary	5,269 (F= 4,027; M=1,242)
Silva et al, 1999 / Rio Grande do Sul	To determine the phlebotomine species of certain areas in the Parque Estadual do Turvo, and to identify the vector species and their infection rate with *Le. (Viannia)* using the polymerase chain reaction (PCR).	P; Ap= Domiciliary; Ap= Rainforest	2,228 (F= 1,090; M=1,138)

Freitas et al, 1989 / Rondônia and Amazonas	The brazilian material (sand flies) is described and illustrated, and compared with specimens of *L. o. nociva* and *L. flaviscutellata* from the same area. Keys to the known taxa of the flaviscutellata complex are presented.	Ap = Rainforest	10,868

Source: Own elaboration.

Table 2 Summary of the outcomes of included studies (n = 35). (NR: not reported; ITS1: Internal transcribed spacer 1 region; DNA: desoxyribonucleic acid; RNA: ribonucleic acid; PCR: Polymerase Chain Reaction; RFLP: restriction fragment length polymorphism; LnPCR: Leishmania-specific nested polymerase chain reaction; MLEE: multilocus enzyme electrophoresis; kDNA: kinetoplast DNA; hsp70: 70 kilodalton heat shock proteins; SSUr RNA: small subunit ribosomal ribonucleic acid; SSUrDNA: Small subunit ribosomal DNA; SL RNA: Spliced Leader; Hgal: Haemophilus gallinarum).

Author, year/ State of Brazil	**Method of *Leishmania* detection (target) *desiccation (Yes/No)**	**Parasite identified / insect collected (Specie or genus)**	**Conclusion**
Araujo-Pereira et al, 2020 / Acre	Multiplex PCR (kDNA) PCR (Hsp70), sequencing	25/ 864. The 25 positive samples, 16 of these samples it was possible to identify the *Leishmania* species: *L. (V.) braziliensis* DNA with 100% identity: *Ev. termitophila* (n = 1); *Ny. antunesi* (n = 1); *Ny. shawi* (n = 2); *Psathyromyia* sp. (n = 1); *Ps. davisi* (n = 1). *L. (V.) braziliensis* with identity values between 89 and 99%: *Brumptomyia* sp. (n = 1); *Ny. umbratilis* (n = 1); *Pi. nevesi* (n = 1); *Ps. davisi* (n = 3); *Ps. hirsutus hirsutus* (n = 1); *richophoromyia* sp. (n = 1). *L. (V.) guyanensis* with 100% identity:*Ny. shawi* (n = 1); *Psychodopygus* sp. (n = 1)	In *Brumptomyia* sp. and *Evandromyia termitophila*, the first report of *Leishmania DNA-detection* is provided in Acre; *Nyssomyia shawi* is implicated as potential vector of *L. (V.) braziliensi*s and *L. (V.) guyanensis* for the first time in Brazil.
Leão et al, 2020 / Rondônia	PCR (kDNA and hsp70)	23 pools / A total of 2817 sand fly females were sorted into 194 pools. kDNA fragment was amplified in 23 pools: 6 from Santa Maria trail (all from canopy) and 17 from Potosi trail (canopy: 15; ground: 2). The minimal infection rate was 0.81% (23/2817). Hsp70 fragment was amplified in 8 pools: 5 pools from the Santa Maria trail and 3 pools from the Potosi trail. Sequencing was successful for 2 samples in which the hsp70 fragment was amplified. Both samples were from the canopy level of the Santa Maria trail; 1 sequence exhibited similarity with *L. braziliensis* (*Lu. davisi* pool) and the other exhibited similarity with *L. naiff* (*Lu. antunesi* pool).	Sand fly fauna is more diverse in the canopy than at ground level. Factors such as blood-meal sources, resting sites, and abiotic components probably contribute to high abundance in the canopy. The results reinforce the possibility that *Lu. antunesi* and *Lu. davisi* participates in *Leishmania* transmission in forest environments and may play an important role in transmission from sylvatic to human hosts.
Sales et al, 2020 / Pernambuco	Fast multiplex Real-time PCR (kDNA)	12 / 97 in *M. migonei* Was tested: *M. migonei (n = 95)* and *Ny. intermedia* (n = 2). *Leishmania* spp.	This multiplex real-time PCR assay represents a novel fast assay for detecting dog, human and *Leishmania* DNA in female sandflies and therefore a tool for assessing the risk of *Leishmania* transmission to these hosts in areas of active transmission. Adding, the proven sand fly vectors are present in the indoor and immediate outdoor environments in indigenous villages of Pernambuco where CL is endemic. The adaptation of sand flies to the indoor environment may be related to the poor housing conditions observed in these villages and the proximity of houses to

			green areas (e.g., crop plantations and forest fragments) (Sales et al., 2019).
Tanure et al, 2020 / Minas Gerais	PCR(ITS1) Desiccation: Yes	13 pools (4,1%) / 4,913 females (47 were individually tested and 4,866 grouped in 311 pools); 4 pools of *Ny. whitmani* positive for *L. amazonensis* (4/311). Seven pools (7/311) it was not possible to determine the *Leishmania* species, due to low quality of the sequences, which contained a high number of ambiguous sites. Therefore, sequences were left as undetermined (*Leishmania* sp.). One pool of *Ps. lloydi* was positive for *L. braziliensis* (1/311) and *Lu. longipalpis* was positive for *Leishmania* sp. (1/311)	The Casa Branca locality has a diverse sand fly fauna with species that have been previously reported in the state of Minas Gerais. The *Ny. whitmani* species is the probable vector of *L. braziliensis* in Casa Branca and may also be involved in the transmission of *L. amazonensis*. The knowledge of the interactions between sand flies and trypanosomatids reported in the *Lu. (Ny.) yuilli yuilliis* study shows that the infection may be occurring in the peridomiciliary environment in the study area. In addition, these results help to understand the dynamics of the leishmaniases transmission cycle in Casa Branca providing support for disease control actions in the region. The presence of non-*Leishmania* trypanosomatids raises an issue that has been neglected and is of great importance, the circulation of these parasites within phlebotomine sand flies.
Uzcátegui et al, 2020 / Pará	PCR-RFLP (TspRI/HgaI endonucleases) Desiccation: Yes	2/1881 samples of *Bi. flaviscutellata* and *Th. Brachipyga* harboring flagellates. 1/NR sample of *Th. braquipyga; 1 Bichromomyia flaviscutellata* and 1 *Trichophoromyia brachipyga* were found naturally infected by flagellates. Only the strain from *Th. brachipyga* was isolated and characterized as *Leishmania (V.) lainsoni*	The urban park surveyed may offer potential risks of disease transmission for which environmental management and continuous entomological surveillance are required. The results also highlighted the medical importance of *Trichophoromyia* species, mainly due to the observations of a possible role of *Th. brachipyga* in the transmission of *L. (V.) lainsoni*. Present and past data also note *Bi. flaviscutellata* as an important vector in Belém. On the other hand, *Ny. antunesi* remains outstanding among the list of potential *L. (V.) lindenbergi* vectors, especially with regard to its observed high frequency and potentially aggressive behavior of females resting on tree bases during the early morning. The monthly fluctuations of these species, however, do not seem to be positively correlated with rainfall.
Pereira-Júnior et al, 2019 / Rondônia	PCR (kDNA and hsp70)	One pool of *Ps. davisi* / A total of 1,755 females were divided into 274 pools representing 35 species. One pool of *Ps. davisi* infected with *L. (V.) braziliensis* (query cover = 100%, identity = 100%, GenBank accession KX573933.1. The infected pool was collected from an FE environment in the municipality of Monte Negro.	The sandflies can switch between blood meal sources in differing environments. The vectors, such as *Ny. antunesi* and *Ps. davisi*, feed on humans and bovines in the PE environments and feed on sylvatic animals, such as anteaters in the FE environments. The sandflies using humans and domestic animals as blood meal sources indicates that the transmission profile might be changing in the PE environments. These findings can be used to enhance the epidemiological surveillance of leishmaniasis in RO.
Carvalho et al, 2018 / Pará	PCR (Mini-exon gene of *Leishmania)* Desiccation: Yes	4 / 1,087 *Lu. flaviscutellata* (4). One of the four *Leishmania* cultures from *Lu. flaviscutellata* was positive, and this was identified as *Le. amazonensis*. In one of the dissected *Lu. flaviscutellata* females captured in December, 2014, it was possible to determine a suprapylarian position of the flagellates suggestive of *Leishmania* (*Leishmania*) species.	The residents of Tracuateua are at risk of infection by *Le. amazonensis* by the bite of *Lu. flaviscutellata* in a sylvatic and occupational transmission pattern.

Chagas et al, 2018 / Amazonas	Multiplex PCR (kDNA)	54 / 670*: Leishmania* sp. in all positive samples. *Nyssomyia anduzei (9), Nyssomyia umbratilis (5), Bichromomyia flaviscutellata (3) Bichromomyia olmeca nociva (3) Evandromyia monstruosa (2) Evandromyia sericea (1) Lutzomyia gomezi (1)Nyssomyia antunesi (1) Psathyromyia aragaoi (1) Psathyromyia dreisbachi (1) Psathyromyia lutziana (1) Psychodopygus amazonensis (2) Psychodopygus ayrozai (2) Psychodopygus chagasi (2) Psychodopygus claustrei (1) Psychodopygus corossoniensis (6) Psychodopygus davisi (4) Psychodopygus hirsutus hirsutus (3) Psychodopygus sq. squamiventris (2) Sciopemyia sordellii (3) Trichophoromyia eurypyga (1)*	There is a high level of species diversity of sand flies in the Tarumã Mirim Rural Settlement. Sand fly species vary across ecotopes and are more abundant in forest ecotopes than peridomicile ecotopes. Some important species implicated as vectors were found in intradomicile and peridomicile locations due to the structural organization of rural settlements and contributing significantly to an increased hazard of ACL transmission.
De Ávila et al, 2018 / Acre	PCR (ITS1)	13 / 206: *L. (V.) braziliensis* was confirmed in twelve sandflies: one *Th. auraensis*, two *Ev. saulensis,* one *Ev. walkeri,* one *Ps. llanosmartinsi,* two *Pi. nevesi*, one *Ps. davisi*, one *Ps. ayrozai*, one *Pa. aragoai*, one *Ev. infraspinosa* and one *Ny. antunesi*. *L. (V.) guyanensis* was confirmed in one *Ps. ayrozai*.	The sand fly fauna found in the present study was composed of 43 species and included known vectors of ATL; The high frequency of *Trichophoromyia auraensis* and *Evandromyia saulensis*, and the detection of *L. (V.) braziliensis* DNA, and *Ps. ayrozai with L. (V.) guyanensis* DNA, indicate that these species could be putative vectors for ATL in this Amazonian region; Investigation of blood sources of sand flies revealed a preference among female sand flies collected in this area for domestic chicken, which may be participating in the population dynamics of these insects; Sand fly abundance was higher in the forest and peridomestic environments in the rural area than in the urban forest (H = 17.9, df =42, P < 0.05).
Vasconcelos dos Santos et al, 2018 / Amapá	PCR-RFLP (RNA polymerase II gene) Desiccation: Yes	13 / 48. Ten isolates from *Ny. umbratilis* exhibited a PCR-RFLP profile identical to that of the *L. (V.) guyanensis.* The PCR-RFLP for the remaining DNA fixed on the glass dissection slides allowed characterizing *L. (V.) guyanensis* from one *Ny. umbratilis* and two *Ev. infraspinosa* specimens.	The ACL transmission in the Oyapock River Basin reflects the Guianan/Amazonian classical ecosystem, where *Ny. umbratilis* remains the main vector. A putative alternative transmission by *Ev. infraspinosa* is possible, but circumstantial parasite ingestion is also likely, as seen with other biologically compatible phlebotomine species cohabiting the same potential *L.* (V.) *guyanensis* reservoir ecotopes.
Araujo-Pereira et al, 2017 / Acre	Multiplex PCR, Dot blot hybridisation and Sequencing (kDNA, IVS6 and hsp70)	12 / 173: *Th. auraensis* (9), *Pressatia* sp. (1) and *Ev. saulensis* (2). *L. (V.) braziliensis* was confirmed in five sandflies: one *Ev. saulensis*, three *Th. auraensis* and one *Pressatia* sp.	*L. (Viannia)* DNA in two *Ev. saulensis,* with the confirmation of *L. (V) braziliensis* in one specimen, correspond to the first record of possible infection associated with this sandfly. The study reveals for the first time, in Brazil, the identification of *T. auraensis* and *Pressatia* sp. infected by *L. (Viannia)* parasites.
Brilhante et al, 2017 / Acre	PCR Dissection: Yes	4 / 708 (specie NR)	This is the first study in Acre state using and comparing both black and white Shannon traps, demonstrating the richness, diversity, and anthropophilic behavior of the phlebotomine species and identifying proven and putative vectors of the etiological agents of leishmaniasis.

Dantas-Torres et al, 2017 / Pernambuco	PCR Real-time and Restriction enzyme analysis (kDNA)	60 / 1,003: *Lu. choti* (30) *L. (V.) braziliensis* was confirmed in thirty *Lu. choti*. The other thirty had no association with any species patter.	This study demonstrates that the temporal dynamics of sand flies is correlated to some extent to climate variables, with some species contrasts. People overnighting in Atlantic rainforest remnants should adopt preventative measures such as the use of repellents on bare skin or clothes and insecticide-treated tents to reduce their exposure to sandflies and other potential disease vectors.
De Souza et al, 2017 / Amapá	Indirect immunofluorescence method (MCAb), Isoenzyme electrophoresis and PCR-RFLP (RNA polymerase II gene) Dissection: Yes	45 / 6,212: *L. (V.) guyanensis* was confirmed in thirteen *Ny. umbratilis*, one *Ny. whitmani* and one *Ny. anduzei*. *L. (V.) naiffi* was confirmed in two *Ps. s. maripaensis* and one *Ny. anduzei*	A review of the literature together with the results of the present study, and other published and unpublished results, indicate that eight phlebotomine species potentially participate in the transmission of *Leishmania (Viannia) naiffi* in Amazonia. *L. (V.) guyanensis* infections in *Ny. umbratilis* and *Ny. anduzei* confirmed them, respectively, as primary and secondary vectors. The circulation of *L. (V.) naiffi* in *Ps. s. maripaensis*, which is highly anthropophilic, raises the possibility of the occurrence of underreported ACL cases related to this parasite. The finding of *L. (V.) naiffi* in *Ny. anduzei* adds yet another vector to the long of suspected vectors of this parasite.
Membrive et al, 2017 / Paraná	PCR Multiplex (kDNA) Dissection: Yes	0/52. No positive test was found for *Leishmania*.	Considering the species of wild animals and sandflies found in São Domingos, the negative test found do not exclude the existence of the *Leishmania* transmission cycle in this preservation area. Even though no positive test was found for *Leishmania*, epidemiological surveillance should be maintained.
Silva et al, 2017 / Pernambuco	PCR	0/490. No positive test was found for *Leishmania*.	The present study contributed towards knowledge of the phlebotomine fauna in an area endemic for ACL in the state of Pernambuco. the rainy period was considered to be the time of highest risk in the study area, given the greater presence of phlebotomines.
Toneli et al, 2017/ Minas Gerais	PCR-RFLP (ITS1) Dissection: Yes	2 / 300: *L. (V.) braziliensis* was confirmed in two *Psy. lloydi*	The data here, combined with vector control efforts, can strengthen the sandfly management plan of Santuário do Caraça. Studies of infection in local mammals, and other fauna, are of great importance for determining hosts / reservoirs and understanding *Leishmania* circulation.
Teles et al, 2016 / Acre	PCR multiplex and PCR-RFLP (kDNA, SL RNA and hsp70)	32 pools / 3,218 (368 pools). *Lu. davisi* (16) and *Lu. laurensis/Lu. ruifrietasi* (16). *L. (V) guyanensis* was confirmed in fourteen pools. *L. (V) braziliensis* was confirmed in six pools	The data from this study demonstrate the great diversity of sandflies species with potential involvement in the leishmaniasis transmission cycle in Assis Brazil; In addition, the abundance of *Lu. davisi* and *Lu. auraensis/Lu. ruifreitasi* with several positive pools for the *L. braziliensis* complex increases the data about vector suspects in the north Brazil.
Miranda et al, 2015 / Pernambuco	PCR (kDNA)	0 / 324. No positive test was found for *Leishmania*.	The present study reinforces this assertion and further indicates that, *Lu. whitmani* is mainly found in the peridomestic environment, even in low-density residential rural areas with mixed forest/agricultural exploitation; It also indicates that in these areas, forest-adapted sand fly species may be

			found in peridomiciliary locations, as houses are frequently constructed nearby the forest; Finally, it points out that the number of sand flies (including *Lu. whitmani*) captured daily is significantly correlated to climatic variables, including saturation deficit, which may represent a useful parameter for studying of sand fly populations in leishmaniasis endemic areas.
Pereira Júnior et al, 2015 / Amazonas	PCR-RFLP and Sequencing (kDNA and hsp70)	14 pools / 1,679 (95 pools)/ *Th. ubiquitalis* (10 pools) and *Ps. davisi* (4 pools). *L. (V.) lainsoni* was confirmed in seven pools of *Th. ubiquitalis*. *L. (V.) shawi* was confirmed in one pool of *Th. ubiquitalis*.	In the Middle Solimões region, the sand fly fauna in terra firme and várzea environments is composed of a few dominant species, and several species with few individuals; The fauna varies between ecotopes, being more abundant in forest ecotopes than in peridomicile ecotopes; The abundance of *Th. ubiquitalis* and its record of presence of *L. (V.) lainsoni* DNA may indicate that this species is a vector for ACL in Tefé Municipality, Amazonas, Brazil.
Rêgo et al, 2015 / Minas Gerais	PCR-RFLP, LnPCR (Nested - PCR) and Sequencing (ITS1 and SSUrDNA gene fragment)	23 pools / 4,760 (1,289 pools): *L. (V) braziliensis* was confirmed in seven pools: one *Martinsmyia minasensis*, one *Micropygomyia capixaba*, one *Mg. peresi* and four *Nyssomyia intermedia*. *L. (V) guyanensis* was confirmed in five pools: one *Lutzomyia renei*, three *Ma. minasensis* and one *Mg. goiana*. *L. infantum chagasi* was confirmed in seven pools: two *Evandromyia lenti*, one *Lu. ischnacantha*, one *Lu. longipalpis*, one *Mg. peresi* and two pool *Ny. intermedia. L. (Viannia)* sp. was confirmed in two pools: one *Lu. longipalpi* and one *Lu. renei*. *L. (L.) amazonensis* was confirmed in two pools: one *Ma. minasensis* and one *Ny. intermedia*.	In this study *Ny. intermedia* was found associated with *Le. infantum chagasi* both in peridomicile areas and among the trails, however, the role of this sand fly species in the epidemiological cycle of this parasite is unclear.
Silva et al, 2014 / Amazonas	PCR (ITS1)	7 pools / 559 (82 pools): *L. (L) amazonensis* was confirmed in four pools: one *Ny. umbratilis*, one *Ny. yulli yulli*, one *Sc. servulolima* and one *Th. ubiquitalis. L. (V) braziliensis* was confirmed in two pools: one *Ev. apurina* and one *Ps. davisi*. *L. (Viannia)* sp.was confirmed in one pool: one *Th. ubiquitalis*.	*Leishmania* infection detected in vectors and suspected species found in this study suggests a role of these species in the transmission cycle of ACL in the Castanheira settlement, Lábrea, Amazonas, and the risk of infection in the study area.
Teles et al, 2013 / Rondônia	PCR (kDNA)	0 / 1,240. No positive test was found for *Leishmania*	From all 53 species captured, four sandflies species were found in the State of Rondonia for the first time: *Brumptomyia brumpti, Lutzomyia tarapacaensis, Lutzomyia melloi and Lutzomyia lenti;* The species *L. whitmani* and *L. davisi* were the most abundant and have proved to be significant vectors of *Leishmania* that cause ACL; The prevalence of these vectors suggests the possibility of transmission in the peridomestic environment. The epidemiologic data points to a significant decrease in the incidence a ACL of about 53% and 43% over the last ten years in Monte Negro and Rondônia and 31% in Brazil.
Thies et al, 2013 / Mato Grosso	LnPCR and Sequencing (SSUrRNA gene fragment)	13 pools / 2,419 (293 pools): *L. (V) braziliensis* and *L. (V) guyanensis* were confirmed in eight pools: six *Lu.*	The natural infection of *L. antunesi* and *L. ubiquitalis by Leishmania* sp. suggests that these species might play

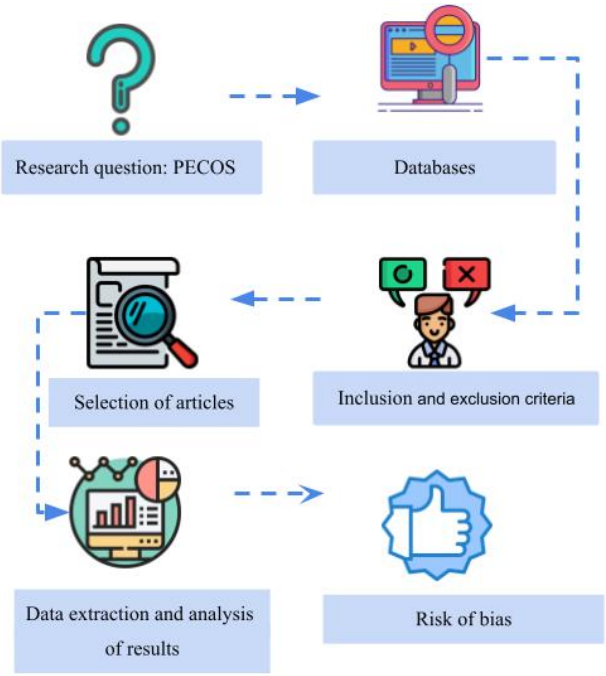
		antunesi and two *Lu. ubiquitalis*. *L. (L) chagasi* was confirmed in one pool: one *Lu. antunesi*	a role in the zoonotic cycle of ACL in Nova Mutum; The presence of *Le. infantum* in *L. antunesi* suggests that there may be a risk of an outbreak of visceral leishmaniasis in Nova Mutum.
Vilela et al, 2013 / Tocantins	PCR multiplex and Dot Blot Hybridisation (kDNA)	(4 pools) / 290 (29 pools). *Leishmania* (Viannia) *braziliensis* was confirmed in three pools of *Ps. complexus* and one pool of *Ps. Ayrozai.*	The rural settlement environment exhibited greater phlebotomine biodiversity than the periurban area. *Ps.complexus* and *Psychodopygus ayrozai* naturally infected with *Leishmania* (Viannia) *braziliensis* were identified. The data identified *Ny. whitmani* as a potential ACL vector in the periurban area, where as *Ps. complexus* was more prevalent in the rural environment associated with settlements.
Quaresma et al, 2012 / Minas Gerais	PCR-RFLP (ITS1 and hsp70)	2 / 38. *L. (V) braziliensis* was confirmed in two sandflies: two *Psychodopygus lloydi*.	The identification of two P. lloydi females infected with *L. braziliensis* suggests that this insect maintains L. braziliensis infections in a sylvatic cycle in Ibitipoca State Park for the following reasons: (i) both individuals were engorged with the blood of rodents/marsupials, (ii) this sandfly species was the most abundant sandfly species in the park, (iii) there are no data on the vector competence of *P. lloydi* and (iv) there have been no reported human cases of cutaneous leishmaniasis in the study area. These results constitute an important step towards validating the use of cytB PCR as a tool for identifying the food sources of naturally feeding female sandflies.
Margonari et al, 2010 / Minas Gerais	PCR-RFLP (kDNA)	63 / 257.*Lu. whitmani* (29); *Lu. neivai* (21); *Lu. cristhenseni* (5); *Lu. pessoai* (5); *Lu. aragaoi,* (1) *Lu. fischeri* (1) *; Lu. lenti* (1); *Lu. lutziana* (1) and *Lu. monticola* (1) *L. (L.) chagasi* was confirmed in one sandflie: one *Lu. whitmani. L. (V.) braziliensis* was confirmed in five sandlfies: one *Lu. fischeri*, one *Lu. monticola,* one *Lu. lutziana,* one *Lu. christenseni* and one *Lu. lenti*	The finding of potential and incriminated vectors naturally infected with *Leishmania* reinforces the need of epidemiologic surveillance in the area.
Saraiva et al, 2010 / Minas Gerais	PCR, PCR - RFLP and Dot-blot hybridization (kDNA) Desiccation: Yes	1 / 243. *Leishmania braziliensis* was confirmed in one Complexo *cortelezzii*	It is important to note that the occurrence of a natural infection is not enough to define a species as a vector in the leishmaniasis cycle.
Souza et al, 2010 / Pará	Specific monoclonal antibodies *Desiccation: Yes	19 / 11.259 *Ps.davisi* (4); *Ps. h. hirsutus* (3); *Lu. umbratilis* (3); *Lu.richardward* (2); *Lu. brachipyga* (2); *Lu. ubiquitalis* (2); *Lu.trinidadenses* (1); *Lu. migonei* (1). *L.* (V.) *braziliensis* was confirmed in two *Ps. davisi*	The phlebotominal fauna of Serra dos Carajás is one of the most diversified in the world, with several species involved in the transmission of enzootic by *Leishmania* spp in wild animals, some of them recognized medical interest and others because they are still clarified. The results of the present study not only confirm the epidemiological importance of some species of phlebotomines found in the fauna of the Serra dos Carajás, as well as to demonstrate the great diversity of local species, in a total of 69 identified, belonging to three genera: *Psychodopygus, Lutzomyia* and *Brumptomyia*.
Brito et al, 2009 / Pernambuco	PCR - RFLP and MLEE (ITS rDNA - internal Transcribed Spacer)	*1/1 L. (V.) braziliensis* was confirmed in one *Lu. whitmani*	This study confirm that transmission cycle complexity and the co-existence of two or more species living in sympatry in the same area can affect

			the level of genetic polymorphism in natural *Leishmania* populations.
Oliveira-Pereira et al, 2006 / Maranhão	PCR * Desiccation: Yes	4 pools / 1,100 (110 pools). *Leishmania* sp. was confirmed in 4 pools of *L. whitmani*	The natural infection rate of sand flies using PCR is sufficient to maintain the endemicity of the infection. PRC was more sensitive than the dissection of sand flies. This study shows the ability of the *L. whitmani* population of Buriticupu to become infected with *Leishmania*, indicating its probable role as LT vector in this municipality.
Souza et al, 2004 / Minas Gerais	PCR	0/398. No positive test was found for *Leishmania*	The highest population density was represented by the species *L. longipalpis* in BH during the period studied. L. longipalpis was the most frequently found in six of the nine regions under study. Analysis on climate influences in phlebotomine sandfly populations in BH showed no statistically significant results. However, there is an increasing trend in the number of phlebotomine insects just after rainy periods. A favorable environment to phlebotomine reproduction associated with the presence of domestic animals in peridomiciliary areas may explain the high number of insects found compared to inner houses under study.
Miranda et al, 2002 / Bahia	PCR and Dot Blot Hybridization (kDNA)	30/335. *L. braziliensis* was confirmed in *Lutzomyia* spp.	The present results clearly show that there is heterogeneity in the spatial distribution of *Leishmania* infected phlebotomines.This study also confirms the large predominance of *L. (N.) whitmani* over other phlebotomine species in this area. There is no indication so far that intradomiciliary sand flies are more likely to transmit leishmaniasis. Combination of directed capture and PCR did not increase the detection of infected sand flies in a large area. However, the use of these two approaches combined with a sectored analysis revealed sectors with a high incidence.
Silva et al, 1999 / Rio Grande do Sul	PCR (kDNA)	3/920 *Lu. pessoai (2) Lu. misionensis (1). L. (Viannia)* in *Lu. pessoai* and *Lu. misionensis.*	Five species that were isolated in the park, *Lu. migonei*, *Lu. pessoai*, *Lu. fischeri, Lu. neivai* and *Lu. shannoni*, are suspected to be vectors of *Le. (Viannia)* elsewhere in Brazil. *Lu. misionensis* was found to be the predominant species in the forest, while *Lu. migonei* and *Lu. pessoai* were the predominant species in domicile and peridomicile areas. Only *Lu. pessoai* and *Lu. misionensis* were found to be infected with *Le. (Viannia)*. Thus, these two vector species do not appear to play an important role in the transmission of leishmaniasis infection to humans.
Freitas et al, 1989 / Rondônia and Amazona	Dissection NR	1/1,345 *L. reducta (1). L. amazonensis* in *L. reducta*.	*L. reducta, L. olmeca nociva* and *L. flaviscutellata* can be distinguished from phlebotomines not included in the flaviscutellata complex by the pigmentation and by the elongated head in the females. The relative abundance of the three simpatric flaviscutellata complex species varies locally and appears to be related with soil drenage. *L. reducta* constituted

about 25% of all phlebotomines captured in Disney traps, appearing not to colonize areas subject to periodic flooding. *L. reducta* is the third species of the flaviscutellata complex to be found infected with *L.amazonensis* in Brazil.

Source: Own elaboration.

Graphical abstracts



***Leishmania* natural infection**	
Leishmania (V.) braziliensis	*Leishmania (v.) naiff*
Leishmania (V.) guyanensis	*Leishmania (V.) shawi*
Leishmania (L.) amazonensis	*Leishmania (V.) lainsoi*

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