Parasites & Vectors

REVIEW

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Identification of asymptomatic *Leishmania* infections: a scoping review

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Abstract

Background: Asymptomatic *Leishmania* infection may play an important role in the transmission of the parasite in endemic areas. At present there is no consensus on the definition of asymptomatic *Leishmania* infection, nor is there a safe and accessible gold standard test for its identification.

Methods: This paper presents a scoping review to summarize definitions of asymptomatic *Leishmania* infection found in the literature, as well as to detail the approach (molecular, serological, cellular, and/or parasitological tests) used by researchers to identify this asymptomatic population. A scoping review of published and gray literature related to asymptomatic *Leishmania* infection was conducted; retrieved citations were screened based on predefined eligibility criteria, and relevant data items were extracted from eligible articles. The analysis is descriptive and is presented using tables, figures, and thematic narrative synthesis.

Results: We conducted a screening of 3008 articles, of which 175 were selected for the full review. Of these articles, we selected 106 that met the inclusion criteria. These articles were published between 1991 and 2021, and in the last 5 years, up to 38 articles were reported. Most of the studies were conducted in Brazil (26%), Spain (14%), India (12%), Bangladesh (10%), and Ethiopia (7%). Of the studies, 84.9% were conducted in the immunocompetent population, while 15.1% were conducted in the immunosuppressed population (HIV, immunosuppressive drugs, and organ transplantation population). We report 14 different techniques and 10 strategies employed by researchers to define asymptomatic *Leishmania* infection in an endemic area.

Conclusions: The definition of asymptomatic *Leishmania* infection is not unified across the literature, but often includes the following criteria: residence (or extended stay) in a *Leishmania*-endemic area, no reported signs/symptoms compatible with leishmaniasis, and positive on a combination of serological, molecular, cellular, and/or parasitological tests. Caution is recommended when comparing results of different studies on the subject of asymptomatic infections, as the reported prevalence cannot be confidently compared between areas due to the wide variety of tests employed by research groups. More research on the importance of asymptomatic immunosuppressed and immunocompetent *Leishmania*-positive populations in leishmaniasis epidemiology is required.

Keywords: *Leishmania*, Leishmaniasis, Asymptomatic, Blood donor, Molecular test, Serological test, Cellular test

Background

Leishmaniasis is considered a neglected tropical disease (NTD). It is a vector-borne infectious disease caused by parasites of the genus *Leishmania*, transmitted by the bite of infected female sand flies [1, 2]. An estimated 12 million cases of leishmaniasis exist worldwide, with 350 million people at risk of infection [3]. Cutaneous

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leishmaniasis (CL) and visceral leishmaniasis (VL) are the most severe clinical forms of the disease; CL affects the skin, while VL affects the internal organs of the infected patient [4]. The evolution of the disease occurs progressively over a period of weeks or even months and is influenced by environmental, parasite-, and host-related factors [5]. VL is fatal in 95% of cases if left untreated [2].

Leishmaniasis is endemic in 98 countries; however, official data underestimate the reality of human leishmaniasis due to the low number of mandatory reporter countries (32/98), the large number of cases that are incorrectly diagnosed, official data being obtained exclusively from passive case detection, and the large, unreported asymptomatic population [3, 6].

Asymptomatic infection represents approximately 20–60% of *Leishmania* spp. infection in endemic areas [7, 8]. Although the asymptomatic population likely represents the highest proportion of infection, there is no agreed definition of the condition or accurate means by which to detect a subject with asymptomatic *Leishmania* infection [9]. Some authors define a subject with asymptomatic infection as a healthy individual living in an endemic area who tests positive on a molecular [polymerase chain reaction (PCR), quantitative PCR (qPCR), or loop-mediated isothermal amplification (LAMP)], serological [direct agglutination test (DAT), enzyme-Linked immunosorbent assay (ELISA) test, rK39-immunochromatographic rapid test (rK39-RDT), immunofluorescence antibody test (IFAT), or Western blot (WB)], or cellular test [leishmanin skin test (LST), interferon gamma release assay (IGRA), whole blood assay (WBA), cell proliferation assay (CPA)]; while others consider a combination of the above tests [10–14].

The asymptomatic population is of vital importance for several reasons. Firstly, they may well serve as a reservoir of parasites, presenting a risk to public health through infection of the phlebotomine vector [15]. Immunosuppression is one of the risk factors that can increase progression to clinical manifestation in asymptomatic subjects. Human immunodeficiency virus (HIV) infection, immunosuppressive drugs, and organ transplantation are the most widely studied risk factors for co-infection with *Leishmania*. HIV infection increases the risk of developing VL by 100–2320 times, while the risk is increased by 20–100 times after treatment with immunosuppressive drugs and after organ transplantation [16, 17]. As such, special attention should be paid to this asymptomatic immunosuppressed (IS) population in endemic areas.

Further interest in the asymptomatic population stems from the mystery surrounding leishmaniasis disease progression. It is well known that a large proportion of those infected with *Leishmania* spp. never demonstrate clinical manifestations of the disease [8]. It has been suggested that progression towards symptomatic VL likely results from a combination of various host, parasite, and sociodemographic factors [18]. A clearer understanding of the manifold factors leading to the development of clinical leishmaniasis could inform the treatment of asymptomatic patients to improve disease outcome, as well as reduce parasite transmission from this potentially significant reservoir [15].

For these myriad reasons, there is an urgent need to establish a specific definition of "asymptomatic *Leishmania* infection," such that future studies may contribute to the development of new leishmaniasis control strategies through standardized and methodical means. To this end, our aims in this study are to outline current approaches used to describe asymptomatic *Leishmania* infection in endemic areas and map out approaches previously used for the study of asymptomatic *Leishmania* infection in blood banks, epidemiological surveys, and through screening of patients in endemic areas. Furthermore, frequently employed definitions of "asymptomatic *Leishmania* infection" and their associated diagnostic tests are discussed, such that we may suggest common usage guidelines concerning these topics to inform future studies in the field.

Methods

Protocol and registration

We developed the protocol for the scoping review in line with the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P). The protocol was developed before beginning the search and was reviewed and approved by all members of the review team.

Eligibility criteria

For this scoping review, we sought to identify primary studies reporting on asymptomatic *Leishmania* infection within human populations in an endemic area (see Additional file 1: Table S1 for the list of endemic countries). Eligible studies could include populations of any age, sex, and health status. Only studies in which a diagnostic technique was employed to identify *Leishmania* in asymptomatic people were considered eligible. We included articles in English, French, Spanish, and Portuguese, and from any period. The eligible study designs included surveillance studies, cross-sectional studies, cohort and case–control studies, and interventional studies. Articles were excluded if they reported on studies of symptomatic *Leishmania* infection, involved only animal populations, or did not include a diagnostic technique. Studies not based on primary data, such as reviews and

modelling studies, were also ineligible for this scoping review.

For our study, we considered subjects as asymptomatically infected with *Leishmania* if they met the following criteria: no signs/symptoms of leishmaniasis (based on clinical examination by a medical professional and/or medical history as declared by the patient), positive on at least one diagnostic test (serological, molecular, cellular, and/or parasitological), and residence (or history of extended stay) in an area of leishmaniasis endemicity.

Information sources

To identify potentially relevant articles, we conducted a detailed search of the PubMed, Web of Science, and LILACS databases. All three bibliographic sources were searched on 11 August 2021. We also conducted a manual search of eligible articles for potentially relevant articles that may have been missed in the bibliographic search.

Search

Our search strategy combined the broad terms "*Leishmania*" and "asymptomatic." We included alternate terms within each concept to improve the sensitivity of the search. No restrictions on language or publication period were included in the search. The initial search strategy was developed by AVIM, CO, and AC, and was reviewed by CFP. The final search strategy was modified via an iterative and consultative process involving all members of the review team. For this scoping review, we did not include gray literature.

The complete search syntax for the PubMed search is presented below, combining Medical Subject Headings (MeSH) and free-text search (Additional file 2: Table S2).

((*Leishmania*[*MeSH Terms*]) *OR* (*leishmania**)) *AND* (((*asymptomatic**) *OR* (*carrier*) *OR* (*blood donor*) *OR* (*subclinical*)))

Selection of sources of evidence

The citations returned from the electronic database searches were imported into EndNote, where duplicate records were identified and deleted. The de-duplicated citations were subsequently imported into COVIDENCE, where further de-duplication was carried out and articles were assessed for eligibility.

AVIM and AC independently screened the titles and abstracts of records to identify potentially relevant studies. Subsequently, AVIM, AC and VW independently read the full text of the potentially relevant articles and selected those that met the eligibility criteria. At each stage of the selection process, two reviewers had to independently agree on the assignment of each article. The agreement was high between voting members, with 95% agreement at the title and abstract stage, and 81–88% at the full-text stage. Disagreement in voting at each stage was resolved by CFP following consultation with the voting pair.

Data charting process

Predefined data items were identified by the review team through a consultative process during the planning of the scoping review and subsequently integrated into an extraction form. The data extraction form was designed and piloted in Excel using some eligible papers and modified as required. One member of the team (AVIM, AC, and VW) conducted the initial data extraction, and a second member crosschecked the extracted data to ensure completeness and accuracy (AVIM, AC, and VW). Discordance in extracted data was resolved by consensus between the reviewers.

Data items

In Table 1 below, we present details of the data items collected as part of the scoping review.

Outcomes and prioritization

The outcome of interest in our review is a description of the common definitions of "asymptomatic *Leishmania* infection" found in the literature, as well as the techniques used for the detection of this population in various endemic areas. No other outcomes are considered for prioritization.

Critical appraisal of individual sources of evidence

We did not conduct a formal critical appraisal of included articles as part of this scoping review, as the aim of this review was to describe the scope of research and not to present pooled study results.

Synthesis of results

We used a descriptive approach in the synthesis and presentation of the scoping review findings. We present an overview of the study selection process through narrative and PRISMA flowchart. A summary of the included articles by year of publication, study country, study setting, study aim(s), and population characteristics is presented in a descriptive table. We subsequently describe how researchers had operationalized and defined asymptomatic *Leishmania* infection and summarize these strategies using a thematic approach. We used a combination of deductive and inductive processes to obtain the definitions based on explicit or implicit case definitions obtained from included articles. We further identified test–test comparison pairs based on a reduced number of eligible articles where two or more tests were used in parallel. *Leishmania* microscopy and culture

Table 1 Data items and characterization

Data item	Characterization
Author	Last name of first author
Year	Year of publication
WHO region	WHO region where study was conducted: European Region, Americas, Eastern Mediterranean Region, South-East Asia, African Region
Country	The country or countries where the study was conducted
Objective of study	As outlined by the authors. This was extracted verbatim and subsequently thematically characterized into analyzable data items, e.g., prevalence survey, test validation, etc.
Population description	Description of the study population, including the methods of selection, e.g., household contacts, blood donors and volunteers
Population size	The size of the sampled population
Leishmania manifestation	The clinical manifestation of *Leishmania* applied to determine symptomatology, including visceral (VL), cutaneous (CL), and post-kala-azar dermal leishmaniasis (PKDL)
Clinical status	The overall clinical status of the study population, including: immunocompetent (IC), immunosuppressed (IS), HIV-infected (HIV), and solid organ transplant (SOT)
History of clinical *Leishmania* disease	Whether a history of previous leishmaniasis was confirmed in the study population
Leishmania species	The species of *Leishmania* under investigation. These include *L. donovani*, *L. infantum*, *L. major*, *L. chagasi*, *L. braziliensis*, *L. amazonensis*, *L. mexicana*, *L. guyanensis*, *L. panamensis*
Diagnostic test used	The diagnostic test used by the investigators, as reported in the manuscript
Definition of asymptomatic disease	As reported directly by the authors where available; alternatively, an implicit definition was inferred from information available in the manuscript

were collectively classified as "parasitology tests," while WBA, LST and CPA tests were categorized as "cellular immunology tests." The other tests included in this study—serological and molecular—were considered individually. Using the suite of network commands in Stata [16], we constructed a network showing comparisons made between tests among eligible studies. We inspected linkages (or interconnectivity) between test types in the network to identify potential gaps in test comparison. Using a combination of narrative synthesis, graphs, and network diagrams, we demonstrate how specific tests were used independently or in combination in the included studies.

Results

Searching for an asymptomatic definition

The diagram used in this scoping review for the selection of articles is shown in Fig. 1, following the Tricco et al. guideline [19]. Initially, the first search in the three chosen databases yielded 4290 articles. After eliminating duplicates, 3008 articles were selected for screening by title and abstract. After the first screening, 175 articles were retrieved. Of these 175 articles, 69 were discarded because they did not meet the inclusion criteria described above. Thus, in total, we selected 106 articles for this scoping review.

The number of studies on the identification of the asymptomatic *Leishmania* populations in endemic areas has increased over the years. Figure 2 shows an exponential increase in the number of studies carried out in 5-year periods. Between 1991 and 1996, only two studies were published describing asymptomatic *Leishmania* infection, while in the current period (2017–2021), as of August 2021, 38 articles had been published.

Articles from 19 countries were identified for this study. Of the 106 total articles, seven studies were conducted in only one country in the World Health Organization (WHO) African region (Ethiopia). Meanwhile, in the WHO South-East Asia Region, 26 studies from three countries (Bangladesh, India, Nepal) were included. In the WHO Eastern Mediterranean Region, 12 studies were included from four countries (Iran, Iraq, Morocco, Tunisia), while in the WHO European Region, 29 studies were included from seven countries (Croatia, France, Greece, Israel, Italy, Spain, Turkey) (Fig. 3). Of the total number of studies, 26% (27/106) were conducted in Brazil, followed by Spain (14%; 15/106), India (12%; 13/106), Bangladesh (8%; 8/106), Ethiopia (7%; 7/106), Iran (7%; 7/106) and Italy (5%; 5/106).

Description of the asymptomatic studies included

Information from the 106 studies describing asymptomatic *Leishmania* infection in endemic areas is described in Table 2. All the studies included were primary studies, where subjects did not manifest any symptoms or signs of the disease. The age of subjects ranged from 2 years to >60 years. Among studies, 94.3% were associated with asymptomatic infection in endemic areas of *L. infantum*

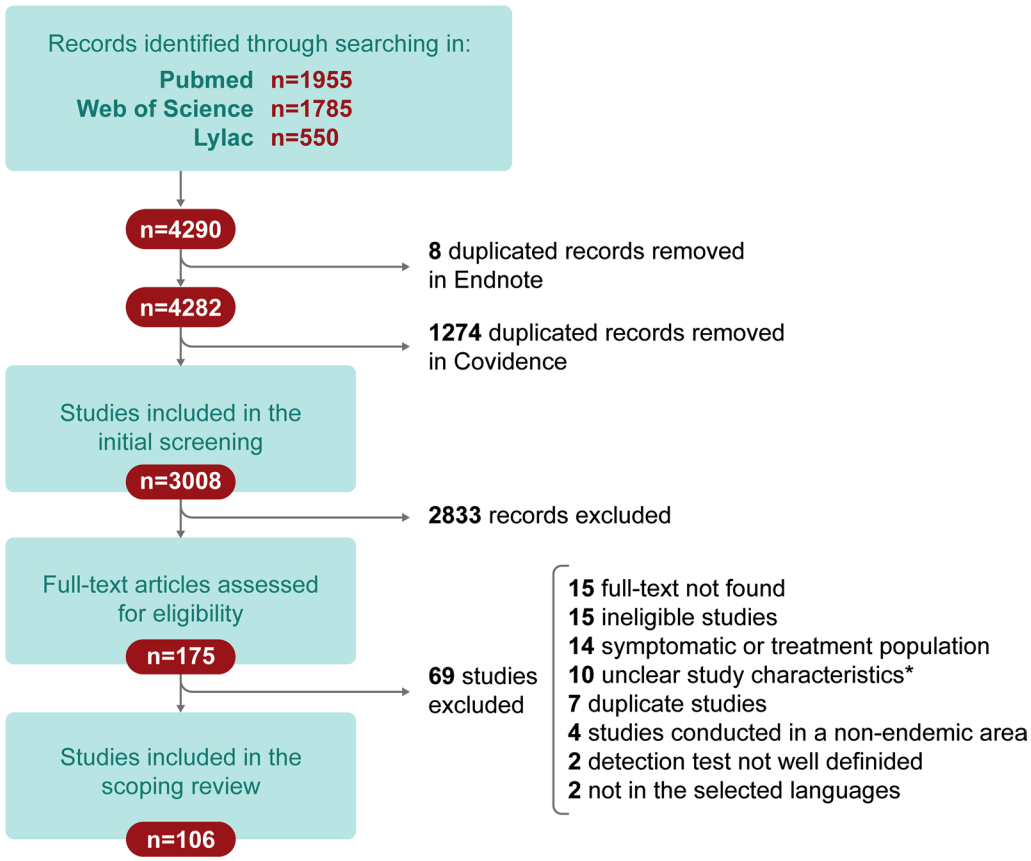

Fig. 1 Flow chart of literature search and screening. *Unclear study characteristics: indicates works where study population inclusion/exclusion criteria, diagnostic methods, and/or overall conceptualization was ambiguous

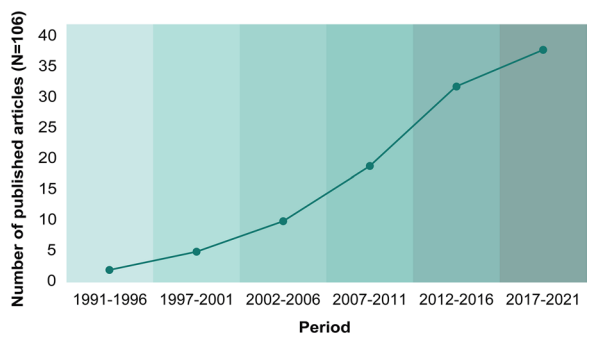

Fig. 2 Yearly frequency of studies published on asymptomatic *Leishmania* infection

and/or *L. donovani*, while 5.7% of studies were performed in the endemic area of *L. major*, *L. braziliensis*, *L. panamensis*, *L. amazonensis*, *L. mexicana*, and *L. guyanensis*.

The clinical status of patients is associated with their immunological status. In this scoping review, the majority of studies (84.9%) were conducted in immunocompetent (IC) subjects. That said, 11.3% of the studies were conducted in HIV patients and 2.8% in solid organ transplant (SOT) recipients, and only 1.9% were identified as having been conducted in IS individuals. The studies in HIV patients were carried out in Spain (n =4), Brazil (n =3), Ethiopia (n =2), France (n =1), Italy (n =1), Iran (n =1), and Morocco (n =1). Meanwhile, the three studies in drug-IS populations were carried out in Spain (n =2) and France (n =1). Finally, the three studies in SOT recipients were carried out in Brazil (n =1), Italy (n =1), and Spain (n =1). Within the 84.9% of works investigating the IC population, 7.8% describe CL, and 2.2% post-kala-azar dermal leishmaniasis (PKDL). We found seven studies in IC populations with various cutaneous manifestations in seven countries: Brazil (n =2), Tunisia (n =2), Colombia (n =1), Peru (n =1), and Venezuela (n =1). Meanwhile, only two studies described PKDL (India and Bangladesh). The vast majority of studies included in this review investigated VL.

A total of 22.6% of included studies were performed in blood banks to determine the prevalence of asymptomatic infection, and 39.6% were performed in volunteer subjects where screening of the population was carried

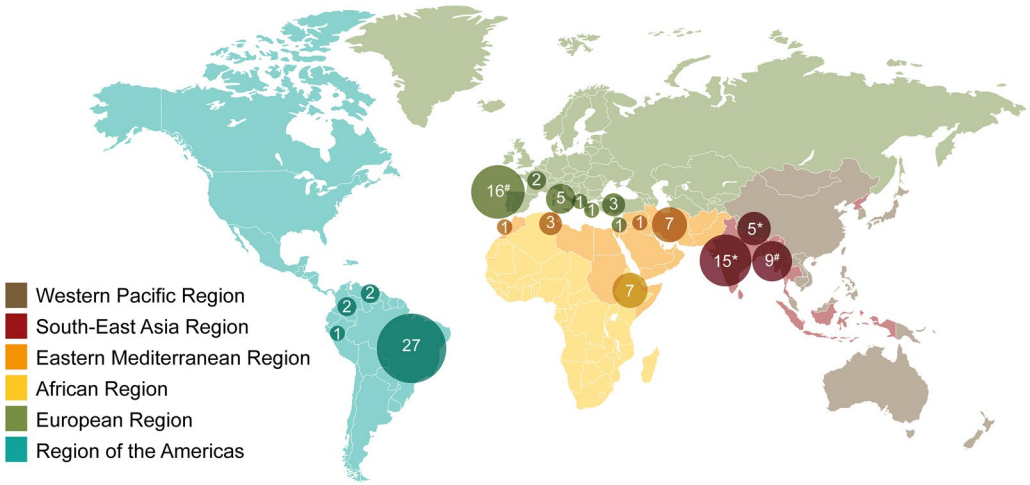

Fig. 3 Distribution by WHO geographic region and countries of studies included in the scoping review. #A study co-conducted in Spain and Bangladesh; *a study co-conducted in India and Nepal

out to evaluate new tests or in clinical trials, or to find markers of exposure or progression. Finally, the remaining 37.7% of studies aimed to determine the prevalence of asymptomatic *Leishmania* infection in subjects who reported close contact with symptomatic leishmaniasis patients (households).

To include patients in blood bank studies, surveys, or clinical trials, knowledge of their previous clinical history is vital. In this scoping review, we found that 9.4% of the studies included subjects with a previous history of leishmaniasis in their asymptomatic group; 59.4% of the studies excluded those subjects who had suffered from leishmaniasis in the past, while 33% did not describe the history of leishmaniasis in the study population.

Tests used for identification of asymptomatic *Leishmania* infection

The inconsistent definition of asymptomatic *Leishmania* infection further demonstrates the lack of consensus in the techniques used. Figure 4 shows an example of the different tissues (blood, serum, urine) used in combination with the tests researchers employed to define the asymptomatic population (Table 2). Parasitological (culture and microscopy), molecular (PCR, qPCR, LAMP), serological (ELISA, RDT, DAT, IFAT, WB, KAtex), and cellular (LST, CPA, WBA) techniques were employed.

Likewise, it was described that the cellular immunity of asymptomatic patients was associated with a Th1-type cellular response, where elevated levels of interferon-gamma (IFN-γ) were produced both in serum and in stimulated plasma and supernatant. It was also found that this group of asymptomatic subjects produced high levels of tumor necrosis factor (TNF), interleukin-2 (IL-2), interferon gamma-induced protein 10 (IP-10, IP-10/CXCL10), monokine induced by gamma interferon (MIG/CXCL9), monocyte chemoattractant protein-1 (MCP-1/CCL2), neopterin, and soluble CD40 ligand (sCD40L), while low levels of IL-10, IL-4, and IL-17 were detected.

Definition of "asymptomatic infection"

As shown in Fig. 4, we report more than 10 different techniques used for the detection of the asymptomatic population in question. "Asymptomatic *Leishmania* infection" frequently describes a subject in a *Leishmania*-endemic area testing positive by a molecular or serological or cellular test, with no signs or symptoms of the disease. However, within the different groups of techniques used, there exist multiple approaches for identification and numerous test combinations for detection of asymptomatic infection. Table 3 shows the different approaches used by researchers to define asymptomatic *Leishmania* infection; 10 different ways to describe these subjects are reported. The first approach involves a combination of four techniques (serological, molecular, cellular, and parasitological). The second and third strategies involve a combination of three techniques (type 2; serological, molecular, and cellular; and type 3; serological, molecular, and parasitological). Meanwhile, types 4 (serological and molecular), 5 (serological and cellular), and 6 (molecular and cellular) used a combination of two techniques. Finally, 33 studies employed a single technique [serological (type 7), molecular (type 8), cellular (type 9), parasitological (type 10)] for the detection of the asymptomatic population in an endemic area.

Table 2 Description of the 106 studies included, divided by WHO regions

Authors	Year	Country	Size (*n*)	Type of leishmaniasis	Clinical manifestation	VL history	Study population	Species	Objective	Ref.
WHO African Region										
Bejano et al.	2021	Ethiopia	1342	VL	IC	nd	Households	*L. donovani*	Prevalence Epidemiology Risk factors	[20]
Tadese et al.	2019	Ethiopia	1099	VL	IC	None	Volunteers	*L. donovani*	Prevalence	[21]
Ayehu et al.	2018	Ethiopia	185	VL	IC	None	Laborers	*L. donovani*	Prevalence Risk factors	[22]
Custodio et al.	2012	Ethiopia	639	VL	IC	None	Households	*L. donovani*	Risk factors Epidemiology	[23]
Gadisa et al.	2012	Ethiopia	605	VL	IC	None	Households	*L. donovani*	Test evaluation	[24]
Griensven et al.	2019	Ethiopia	511	VL	HIV	None	Volunteers	*L. donovani*	Prevalence Incidence Disease progression	[13]
Adriaensen et al.	2018	Ethiopia	35	VL	HIV	Yes	Volunteers	*L. donovani*	Immunological biomarkers Test evaluation	[25]
WHO South-East Asia Region										
Basnyat et al.	2021	Nepal	189	VL	IC	None	Households	*L. donovani*	Prevalence Leishmaniasis contacts Epidemiology	[26]
Cloots et al.	2021	India	94	VL	IC	None	Volunteers	*L. donovani*	Test evaluation	[27]
Owen et al.	2021	Bangladesh	720	VL	IC	None	Households	*L. donovani*	Test evaluation Leishmaniasis contacts	[28]
Johanson et al.	2020	India	109	VL	IC	None	Households	*L. donovani*	Prevalence Leishmaniasis contacts Epidemiology	[29]
Chakravarty et al.	2019	India	1606	VL	IC	nd	Households	*L. donovani*	Test evaluation Disease progression	[18]
Mondal et al.	2019	Bangladesh	200	VL	IC	None	Volunteers	*L. donovani*	Immunological biomarkers Disease progression Test evaluation	[30]

Table 2 (continued)

Authors	Year	Country	Size (*n*)	Type of leishmaniasis	Clinical manifestation	VL history	Study population	Species	Objective	Ref.
Singh et al.	2018	India	64	VL	IC	nd	Volunteers	*L. donovani*	Immunological biomarkers	[31]
Kaushal et al.	2017	India	246	VL	IC	Yes	Volunteers	*L. donovani*	Prevalence	[32]
Saha et al.	2017	India	2603	VL	IC	None	Volunteers	*L. donovani*	Prevalence	[33]
									Disease progression	
Banu et al.	2016	Bangladesh, Australia	706	VL	IC	None	Blood donors and volunteers	*L. donovani*	Test evaluation	[34]
Banu et al.	2016	Bangladesh	257	VL	IC	None	Households	*L. donovani*	Prevalence	[35]
									Leishmaniasis contacts	
Das et al.	2016	India	5144	VL and PKDL	IC	None	Households	*L. donovani*	Leishmaniasis contacts	[36]
									Disease progression	
									Epidemiology	
Timilsina et al.	2016	Nepal	507	VL	IC	None	Blood donors	*L. donovani*	Prevalence	[37]
Vallur et al.	2016	Bangladesh	104	VL	IC	None	Households	*L. donovani*	Test evaluation	[38]
Picado et al.	2014	India and Nepal	510	VL	IC	None	Households	*L. donovani*	Risk factors	[39]
									Disease progression	
									Epidemiology	
Sudarshan et al.	2014	India	130	VL	IC	nd	Households	*L. donovani*	Test evaluation	[40]
									Disease progression	
Sudarshan et al.	2014	India	1469	VL	IC	nd	Households	*L. donovani*	Test evaluation	[41]
									Disease progression	
Huda et al.	2013	Bangladesh	1195	VL	IC	None	Blood donors	*L. donovani*	Prevalence	[42]
Srivastava et al.	2013	India	286	VL	IC	None	Households	*L. donovani*	Prevalence	[43]
									Test evaluation	
Ostyn et al.	2011	India and Nepal	9034	VL	IC	None	Volunteers	*L. donovani*	Disease progression	[44]
Topno et al.	2010	India	335	VL	IC	Yes	Households	*L. donovani*	Prevalence	[45]
									Disease progression	
Bhattarai et al.	2009	Nepal	231	PKDL	IC		Households	*L. donovani*	Test evaluation	[46]
Gidwani et al.	2009	India	870	VL	IC	None	Households	*L. donovani*	Prevalence	[47]
									Leishmaniasis contacts	
									Disease progression	
									Epidemiology	

Table 2 (continued)

Authors	Year	Country	Size (*n*)	Type of leishmaniasis	Clinical manifestation	VL history	Study population	Species	Objective	Ref.
Sinha et al.	2008	India	172	VL	IC	None	Households	*L. donovani*	Test evaluation Leishmaniasis contacts	[48]
Bern et al.	2007	Bangladesh	1379	VL	IC	None	Households	*L. donovani*	Incidence Risk factors	[49]
Chowdhury et al.	1993	Bangladesh	17 826	VL	IC	nd	Households	*L. donovani*	Test evaluation Prevalence	[50]
WHO Eastern Mediterranean Region										
Mody et al.	2019	Iraq	200	VL	IC	Yes	Soldiers	*L. infantum*	Prevalence Risk factors	[51]
Gigloo et al.	2018	Iran	617	VL	IC	None	Households	*L. infantum*	Prevalence Risk factors	[52]
Asfaram et al.	2017	Iran	600	VL	IC	None	Blood donors	*L. infantum*	Prevalence	[53]
Sarkari et al.	2015	Iran	2003	VL	IC	nd	Blood donors	*L. infantum*	Prevalence	[54]
Mohammadiha et al.	2013	Iran	82	VL	IC	None	Volunteers	*L. infantum*	Test evaluation	[55]
Sassi et al.	2012	Tunisia	119	VL and CL	IC	None	Volunteers Households	*L. infantum* and *L. major*	Test evaluation	[56]
Saghrouni et al.	2012	Tunisia	94	VL	IC	None	Households	*L. infantum* and *L. major*	Frequency Leishmaniasis contacts	[57]
Alborzi et al.	2008	Iran	388	VL	IC	None	Volunteers		Prevalence Test evaluation	[58]
Fakhar et al.	2008	Iran	802	VL	IC	Yes	Households	*L. infantum*	Prevalence	[59]
Sassi et al.	1999	Tunisia	45	CL	IC		Volunteers	*L. major*	Immunological biomarkers Test evaluation	[60]
Echchakery et al.	2018	Morocco	200	VL	HIV	None	Volunteers	*L. infantum*	Prevalence	[61]
Rezaei et al.	2018	Iran	251	VL	HIV	None	Volunteers	*L. infantum*	Prevalence	[62]
WHO European Region										
Molina et al.	2020	Spain	50	VL	IC	None	Blood donors	*L. infantum*	Epidemiology	[63]
Ortalli et al.	2020	Italy	240	nd	IC	None	Blood donors	*L. infantum*	Prevalence	[11]

Table 2 (continued)

Authors	Year	Country	Size (*n*)	Type of leishmaniasis	Clinical manifestation	VL history	Study population	Species	Objective	Ref.
Aliaga et al.	2019	Spain	1260	VL	IC	nd	Blood donors	*L. infantum*	Prevalence Risk factors Epidemiology	[64]
Ibarra-Meneses et al.	2019	Spain	805	VL	IC	None	Volunteers	*L. infantum*	Prevalence Risk factors	[12]
Ibarra-Meneses et al.	2017	Spain	40	VL	IC	nd	Blood donors	*L. infantum*	Immunological biomarkers	[65]
Ibarra-Meneses et al.	2017	Spain and Bangladesh	305 and 25	VL	IC	None	Blood donors and volunteers	*L. infantum* *L. donovani*	Immunological biomarkers	[66]
Ibarra-Meneses et al.	2016	Spain	47	VL	IC	nd	Blood donors	*L. infantum*	Immunological biomarkers	[67]
Pérez-Cutillas et al.	2015	Spain	657	VL	IC	nd	Blood donors	*L. infantum*	Prevalence Spatial distribution Epidemiology	[68]
Ates et al.	2013	Turkey	343	VL	IC	nd	Blood donors	*L. infantum*	Prevalence Test evaluation	[69]
Sisko-Kraljevic et al.	2013	Croatia	2035	VL	IC	nd	Volunteers	*L. infantum*	Prevalence	[70]
Ates et al.	2012	Turkey	188	VL	IC	None	Blood donors	*L. infantum*	Prevalence Test evaluation	[71]
Riera et al.	2008	Spain	1437	VL	IC	None	Blood donors	*L. infantum*	Prevalence	[72]
Scarlata et al.	2008	Italy	1449	VL	IC	None	Blood donors	*L. infantum*	Prevalence	[73]
Sakru et al.	2007	Turkey	82	VL	IC	nd	Volunteers	*L. infantum*	Prevalence	[74]
Papadopoulou et al.	2005	Greece	1200	VL	IC	None	Volunteers	*L. infantum*	Prevalence	[75]
Riera et al.	2004	Spain	656	VL	IC	nd	Blood donors	*L. infantum*	Test evaluation	[76]
Adini et al.	2003	Israel	2580	VL	IC	nd	Households	*L. donovani*	Prevalence	[77]
Fichoux et al.	1999	France	565	VL	IC	None	Blood donors	*L. infantum*	Prevalence	[78]
Federico et al.	1991	Italy	591	VL	IC	None	Blood donors	*L. infantum*	Prevalence	[79]
Botana et al.	2019	Spain	82	VL	HIV	None	Volunteers	*L. infantum*	Immunological biomarkers	[80]
Ena et al.	2014	Spain	179	VL	HIV	None	Volunteers	*L. infantum*	Prevalence	[81]
Colomba et al.	2009	Italy	145	VL	HIV	Yes	Volunteers	*L. infantum*	Prevalence Infection markers	[82]

Table 2 (continued)

Authors	Year	Country	Size (*n*)	Type of leishmaniasis	Clinical manifestation	VL history	Study population	Species	Objective	Ref.
Garcia-Garcia et al.	2006	Spain	92	VL	HIV	None	Volunteers	*L. infantum*	Prevalence	[83]
									Test evaluation	
Pineda et al.	1998	Spain	291	VL	HIV	Yes	Volunteers	*L. infantum*	Prevalence	[84]
									Risk factors	
Botana et al.	2021	Spain	94	VL	IS	None	Volunteers	*L. infantum*	Immunological biomarkers	[85]
									Prevalence	
Guillen et al.	2020	Spain	192	VL	IS	None	Volunteers	*L. infantum*	Prevalence	[86]
									Disease progression	
Mary et al.	2006	France	111	VL	IC, HIV, and IS	None	Volunteers	*L. infantum*	Test evaluation	[87]
Comai et al.	2021	Italy	119	VL	SOT	None	Volunteers	*L. infantum*	Prevalence	[17]
Elmahallawy et al.	2015	Spain	625	VL	SOT	None	Volunteers	*L. infantum*	Prevalence	[88]
Region of the Americas										
Silva et al.	2020	Brazil	500	VL	IC	nd	Blood donors	*L. infantum*	Prevalence	[89]
								L. braziliensis		
Porcino et al.	2019	Brazil	132	VL	IC and VL	nd	Volunteers	*L. infantum*	Test evaluation	[90]
									Immunological biomarkers	
Ferreira-Silva et al.	2018	Brazil	608	VL	IC	None	Blood donors	*L. infantum*	Prevalence	[91]
Marques et al.	2017	Brazil	935	VL	IC	None	Households	*L. chagasi*	Prevalence	[92]
									Risk factors	
Medeiros et al.	2017	Brazil	33	VL	IC	None	Volunteers	*L. infantum*	Test evaluation	[93]
Braga et al.	2015	Brazil	176	CL	IC	None	Blood donors	*L. braziliensis*	Prevalence	[94]
Fukutani et al.	2014	Brazil	700	VL	IC	None	Blood donors	*L. infantum*	Prevalence	[95]
								L. amazonensis		
Franca et al.	2013	Brazil	430	VL	IC	None	Blood donors	*L. chagasi*	Prevalence	[96]
Silva et al.	2013	Brazil	149	VL	IC	Yes	Volunteers	*L. chagasi*	Disease progression	[97]
Añez et al.	2012	Venezuela	1036	VL	IC	None	Households	*L. infantum*	Prevalence	[98]
Santos et al.	2012	Brazil	1875	VL	IC	nd	Households	*L. infantum*	Disease progression	[99]
Lima et al.	2012	Brazil	345	VL	IC	nd	Households	*L. chagasi*	Prevalence	[100]
Carneiro et al.	2011	Brazil	1604	VL	IC	nd	Households	*L. infantum*	Test evaluation	[101]
									Disease progression	
									Epidemiology	
Silva et al.	2011	Brazil	246	VL	IC	None	Volunteers	*L. chagasi*	Disease progression	[102]

Table 2 (continued)

Authors	Year	Country	Size (*n*)	Type of leishmaniasis	Clinical manifestation	VL history	Study population	Species	Objective	Ref.
Crescente et al.	2009	Brazil	946	VL	IC	nd	Households	*L. chagasi*	Prevalence	[103]
Romero et al.	2009	Brazil	1017	VL	IC	None	Volunteers	*L. chagasi*	Test evaluation	[104]
Viana et al.	2008	Brazil	138	VL	IC	None	Volunteers	*L. chagasi*	Prevalence	[105]
									Immunological biomarkers	
									Leishmaniasis contacts	
Oliveira et al.	2008	Brazil	220	VL	IC	nd	Households	*L. chagasi*	Prevalence	[106]
									Leishmaniasis contacts	
Nascimento et al.	2006	Brazil	1016	VL	IC	Yes	Households	*L. chagasi*	Immunological biomarkers	[107]
Moreno et al.	2006	Brazil	1604	VL	IC	nd	Households	*L. chagasi*	Prevalence	[108]
									Test evaluation	
Nascimento et al.	2005	Brazil	1520	VL	IC	nd	Volunteers	*L. chagasi*	Prevalence	[109]
Braz et al.	2002	Brazil	168	VL	IC	None	Household	*L. chagasi*	Test evaluation	[110]
Caldas et al.	2001	Brazil	648	VL	IC	Yes	Households	*L. chagasi*	Prevalence	[111]
									Risk factors	
Corredor et al.	1999	Colombia	1140	VL	IC	None	Households	*L. chagasi*	Prevalence	[112]
							Indigenous		Risk factors	
									Epidemiology	
Guarin et al.	2006	Colombia	11	CL	IC	None	Volunteers	*L. panamensis*	Immunological biomarkers	[113]
								L. amazonensis	Test evaluation	
Torrellas et al.	2020	Venezuela	841	CL	IC	nd	Households	*L. mexicana*	Prevalence	[14]
								L. braziliensis		
								L. guyanensis		
Arraes et al.	2008	Brazil	130	CL	IC	nd	Households	*L. braziliensis*	Prevalence	[114]
									Leishmaniasis contacts	
Best et al.	2018	Peru	28	CL	IC	nd	Households	*L. braziliensis*	Immunological biomarkers	[115]
									Disease progression	
Guedes et al.	2021	Brazil	487	VL	HIV	None	Volunteers	*L. infantum*	Prevalence	[116]
Cunha et al.	2020	Brazil	240	VL	HIV	None	Volunteers	*L. infantum*	Frequency	[117]

Table 2 (continued)

Authors	Year	Country	Size (*n*)	Type of leishmaniasis	Clinical manifestation	VL history	Study population	Species	Objective	Ref.
Orsini et al.	2012	Brazil	381	VL	HIV	nd	Volunteers	*L. infantum*	Prevalence	[118]
Clemente et al.	2014	Brazil	67	VL	SOT	None	Volunteers	*L. infantum*	Prevalence	[119]

VL visceral leishmaniasis; *CL* cutaneous leishmaniasis; *PKDL* post-kala azar dermal leishmaniasis; *IC* immunocompetent; *HIV* human immunodeficiency virus; *SOT* solid organ transplant; *nd* not defined; *Ref.* reference

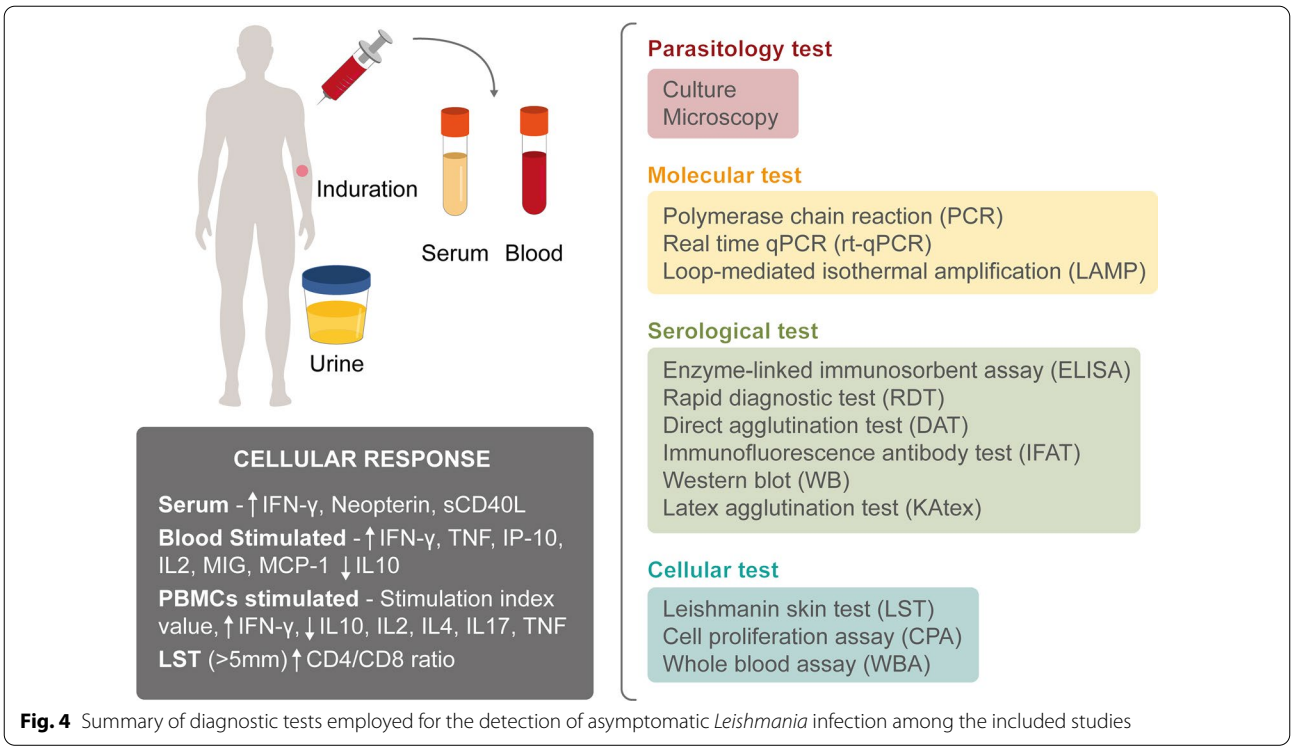

Fig. 4 Summary of diagnostic tests employed for the detection of asymptomatic *Leishmania* infection among the included studies

Markers for asymptomatic infection

Fifty percent of the studies included in this scoping review used an ELISA for identification of asymptomatic *Leishmania* infection, followed by PCR (40%), RDT (35%), IFAT (30%), DAT (29%), and LST (22%). To determine how multiple tests are applied in parallel, we identified 78 eligible studies where at least two tests were used on either the entire population or a subset thereof. Inspection of the network (Fig. 5) showed a high degree of interconnectivity between several of the tests. The highest density of interconnectivity was observed for DAT, RDT, IFAT, PCR, and ELISA, where links were found to all other tests. LAMP was the test method with the least interconnectivity within the network, linking only with ELISA, RDT, IFAT, and PCR once each.

A combination of serological and molecular techniques was the most common combination of detection approaches among the studies included in this scoping review. However, it is important to note that the results mentioned above do not consider the antigen or target used. Figure 6a summarizes the diversity of commercial brands that exist for the rK39 rapid diagnostic test (rK39-RDT). Sixty-three percent of the studies that employed RDT used the rK39 Kalazar *Detect*™ rapid test (InBios), while 11% used IT Leish (BioRad), 6% used the SD BIOLINE *Leishmania* Ab Test (Bioline/Abbott), 6% used OnSite *Leishmania* IgM/IgG Combo test (CTK Biotech, Inc.), 3% used the Leti Laboratories test, and 11% of articles did not describe the commercial brand in question. Figure 6b shows the diversity of antigens employed for the detection of antibodies by ELISA. Thirty-four percent of the studies that used ELISA for identification of the asymptomatic population used soluble *Leishmania* antigen (SLA), another 34% used the rK39 antigen, while 16% used crude *Leishmania* antigen (CSA), 4% used recombinant kinesin 26 (rK26) antigen, 3% used *Leishmania* promastigotes, and 1% used recombinant kinesin 28 (rK28), rTR18, and rKR95 antigen. For the molecular techniques described (Fig. 6c), nine different targets were identified. Of these, 57% of studies used kinetoplast DNA as their target (kDNA), 21% the small subunit *18S rRNA* gene (*ssu* 18S rRNA), and 11% and 3% used internal transcribed spacer (ITS) 1 and 2, respectively. Meanwhile, 1% of studies employed the glucose-6-phosphate dehydrogenase gene (*g6pd* gene), mini-exon repeat, REPL repeat, DNA polymerase alpha (DNApα), and the telomeric region.

Discussion

Our scoping review included a total of 106 articles from 19 countries in five of six different WHO regions. There has been a marked increase in studies conducted on the subject of asymptomatic *Leishmania* infections in recent years (2017–2021) versus past decades, possibly due to increased awareness of their potential significance in the epidemiology of leishmaniasis. Most of the

Table 3 Strategies employed by researchers to detect asymptomatic *Leishmania* infection

Type	Test(s) used	No. of studies	References
1	Serological and molecular and cellular and parasitological	2	[72, 76]
2	Serological and molecular and cellular	13	[12, 18, 27, 51, 58, 63, 66, 80, 83, 85, 97, 100, 105]
3	Serological and molecular and parasitological	7	[35, 55, 61, 69, 78, 90, 119]
4	Serological and molecular	31	[11, 13, 17, 28, 32, 34, 41–43, 45, 46, 52–54, 59, 62, 64, 68, 73, 82, 86, 87, 91, 93, 95, 98, 99, 101, 108, 116–118]
5	Serological and cellular	14	[20, 21, 23–25, 29, 31, 49, 102, 103, 109–112]
6	Molecular and cellular	2	[14, 67]
7	Positive serological test(s) only	28	[22, 26, 30, 33, 36–39, 44, 47, 48, 50, 57, 70, 71, 74, 75, 77, 79, 81, 88, 92, 94, 96, 104, 106, 107, 114]
8	Positive molecular test(s) only	2	[41, 89]
9	Positive cellular test(s) only	5	[56, 60, 65, 113, 115]
10	Positive parasitological test only	1	[84]

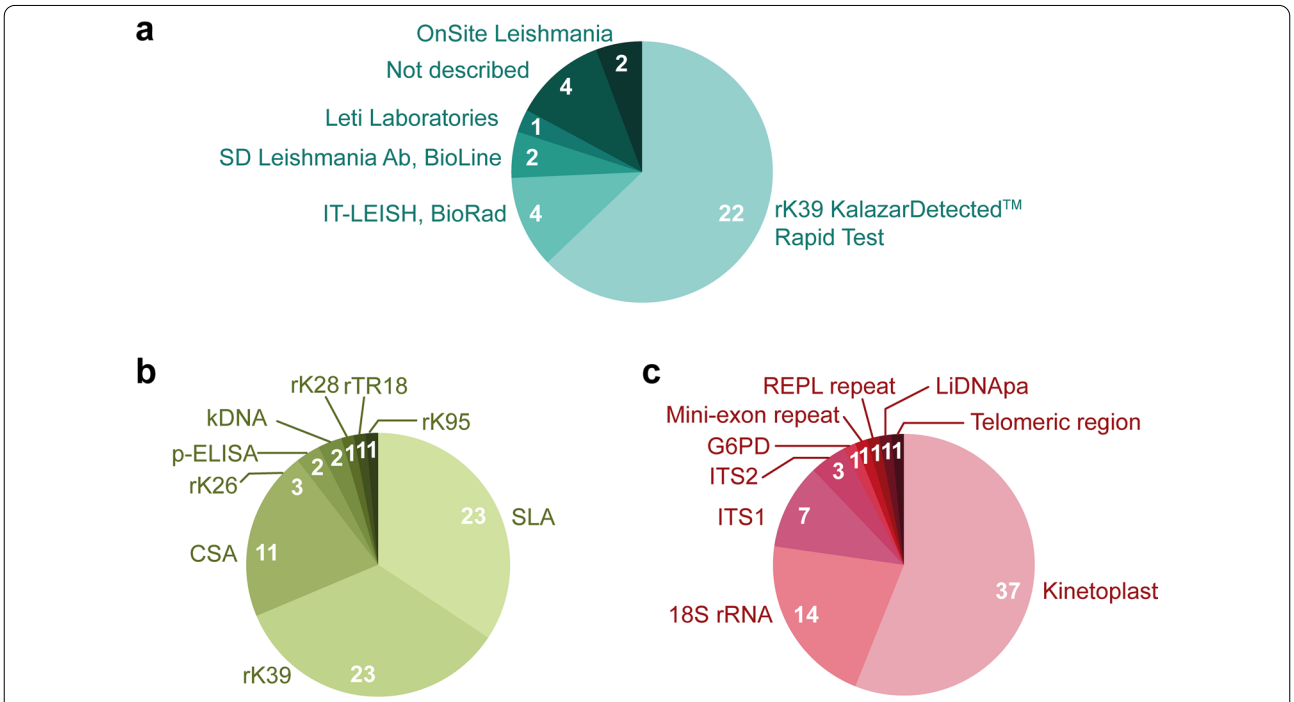

Fig. 5 Studies included in the scoping review using **a** rapid diagnostic tests (RDT), **b** ELISA, and **c** molecular tests for detection of asymptomatic *Leishmania* infection. Pie slice size represents the percentage of tests of each type with the indicated target/brand, while numerals denote the number of papers represented by each slice

studies included in our review were conducted in Brazil (26%) and India (12%); this is likely because leishmaniasis is very widespread in these countries, which constitute two of the six countries responsible for more than 90% of VL cases around the world [3]. The vast majority (84.9%) of studies included in this review explore asymptomatic *Leishmania* infection in IC populations. Different mathematical modeling studies have shown that this asymptomatic IC population is less infective than the population with active disease, and their role in disease transmission is still under investigation [15, 63]. The issue remains of major concern and could have a substantial impact on the spread of this parasite [63, 91].

Notably, leishmaniasis is of much greater risk to IS populations, including HIV-positive individuals, SOT recipients, and patients under treatment with

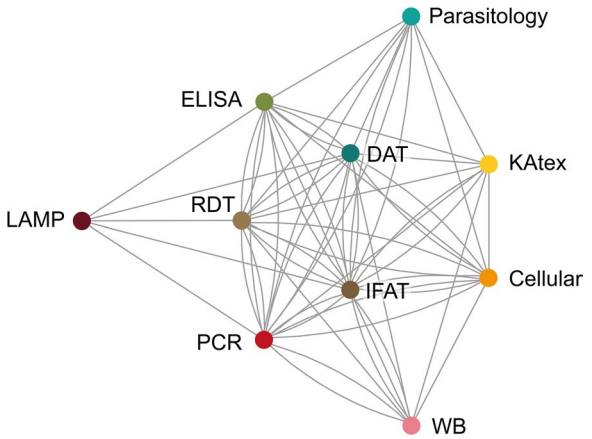

Fig. 6 Network demonstrating comparisons of various tests employed for the detection of asymptomatic *Leishmania* infection within articles included in the scoping review. In the network diagram, the nodes represent the test type and the edges (lines) indicate that two test types have been compared in at least one study

immunosuppressive drugs. HIV/*Leishmania* co-infections more often lead to clinical VL, and with greater severity [117, 120]. Indeed, it has previously been demonstrated that HIV-infected individuals with asymptomatic *Leishmania* infection may transmit the parasite to sand flies, leading to further spread of infection [63, 121]. That said, in many studies, the number of participants is too low to confirm results. Of note, the majority of the studies performed in an HIV-positive population included in this scoping review were conducted in the WHO European Region, the area where most HIV cases have been reported [120]. However, over the years, there has been an increase in HIV cases in other *Leishmania*-endemic areas, such as Brazil, East Africa, and India [13, 116].

Given the likely significance of the asymptomatic IS population in *Leishmania* epidemiology as described above, knowing the prevalence of HIV infection (and furthermore, exploring biomarkers for VL progression) in *Leishmania*-endemic areas becomes ever more important; interestingly, the studies included in our review used up to 13 tests and six different approaches to define infection in this population (see Table 3). The large number of tests and strategies employed underscores the difficulty in defining asymptomatic infection in HIV patients in endemic areas. Interestingly, one research group used only a single parasitological test for this purpose. Pineda et al. describe an asymptomatic HIV patient as one in whom amastigotes are detected in bone marrow aspirate samples [84]. This is in fact the gold standard for diagnosis of *Leishmania*; however, bone marrow aspirate is an invasive approach [122]. Currently, in the WHO road map, minimally invasive techniques that enable detection of this population (and therefore safe and efficient establishment of new control measures) are prioritized [123].

With respect to populations under treatment with immunosuppressive drugs, despite the low number of studies reported ($n = 3$), seven different tests and two different strategies were employed for detection of the asymptomatic population [85–87]. This same pattern was found with SOT recipients: five different tests and three different strategies were used in the three studies reported in this scoping review [17, 88, 119]. These data confirm the lack of consensus on defining and detecting asymptomatic infection in these populations.

In total, this scoping review identified 14 different tests used for detection of parasites, parasite load, antibodies/antigens, and cellular immune response, resulting in nine different overall approaches for defining asymptomatic infections in IC populations. There is a complex relationship between the various tests used for the detection of asymptomatic populations, as demonstrated by the interconnectivity in our network. The nine strategies employed can be separated into two main categories: strategies 1–6 involved independent combinations of serological, molecular, cellular, and/or parasitological tests to identify the asymptomatic cohort; on the other hand, strategies 7, 8, and 9 involved the use of a single test type to detect asymptomatic individuals. These two very different, wide categories broaden the detection range, leading to a lack of consensus concerning the identification of the asymptomatic population in *Leishmania*-endemic areas. Furthermore, the use of different strategies makes it difficult to compare the same population in the same (or different) endemic areas. Importantly, this complication lies not only in the large number of tests employed, but also in the numerous targets (molecular tests) or antigens (serological and cellular tests) used by each group. We report the use of more than six types of rapid test (rK39-RDT) from different manufacturers, and the effectiveness of these commercial tests varies between regions [124]. Moreover, nine different antigens were utilized to detect antibodies using ELISA alone [125], and nine different targets were employed to identify the parasite with molecular tools [126]. This substantial variation in detection methods coupled with the plethora of definitions of "asymptomatic *Leishmania* infection" makes an accurate determination of prevalence in any region near impossible.

Common usage guidelines

Standardization of the term "asymptomatic *Leishmania* infection" is key to improving the outcome of future studies and allowing accurate comparison between the results

Table 4 Gaps and opportunities associated with asymptomatic *Leishmania* infection

Gaps
Lack of consensus regarding definition of asymptomatic infection
Lack of consensus regarding optimal technique for identification of asymptomatic population
Large variety of test targets and antigens employed by different research groups
Lack of knowledge pertaining to the potential role of asymptomatic individuals in *Leishmania* disease transmission and epidemiology
Lack of knowledge pertaining to the factors associated with development of clinical leishmaniasis by individuals previously considered to be asymptomatically infected
Opportunities
Establish a standard definition of "asymptomatic *Leishmania* infection"
Determine the optimal technique for identification of the asymptomatic population (technique, target/antigen)
Determine the true prevalence of asymptomatic *Leishmania* infection in different regions
Determine the true role of asymptomatic *Leishmania*-infected subjects (both immunocompetent and immunosuppressed) in transmission of leishmaniasis
Establish objective, quantifiable markers associated with the development of clinical leishmaniasis by previously asymptomatically infected individuals (differentiate subclinical and asymptomatic infections)
Determine the principal risk factors related to development of clinical leishmaniasis

of different works. According to the literature, subjects are often considered as asymptomatically infected with *Leishmania* under the following conditions: residence (or extended stay) in a *Leishmania*-endemic area, no reported signs/symptoms compatible with leishmaniasis, and positive on a combination of serological, molecular, cellular, and/or parasitological tests. We consider this an appropriate definition. While a medical history of leishmaniasis may complicate interpretation of certain tests, we feel it does not preclude the development of an asymptomatic infection if the subject was previously considered "cured." More research regarding how many years following a reported VL episode a patient can be considered "asymptomatic" is necessary; as such, it is recommended that the conditions of a study population with VL history be explicitly stated. Furthermore, we recommend caution when comparing results of different studies on the subject of asymptomatic infections, as the reported prevalence cannot confidently be compared between areas due to the wide variety of tests employed by research groups. With respect to future studies, we suggest that researchers use the most sensitive and specific test available to them, and interpret their results within the framework of other studies employing the same technique and target. We recommend a minimum of two different detection methods employed in parallel for identification of asymptomatic *Leishmania* infections among a study population.

Of note, for this work, we did not analyze the results/methodology of each piece of literature included in the review; this was beyond the scope of our study. There may therefore be limitations with respect to the quality of the methodology employed in each individual article. In the future, we will perform a meta-analysis in order to compare the sensitivity and specificity of different diagnostic tests employed by research groups for the detection of asymptomatic *Leishmania*-infected populations in endemic areas (Table 4).

Conclusions

Asymptomatic *Leishmania* infection remains poorly understood; the lack of baseline tests for its detection means that its prevalence is likely underestimated, and its epidemiological role remains unknown. This scoping review was performed in order to inform researchers of the different approaches that exist for identification of asymptomatic *Leishmania* infection. It also highlights the need to standardize the definition of this population in order to reach a consensus for future work strategies in endemic areas, especially in IS populations.

Abbreviations

CL: Cutaneous leishmaniasis; DAT: Direct agglutination test; CPA: Cell proliferation assay; CSA: Crude *Leishmania* antigen; DNApα: DNA polymerase alpha; ELISA: Enzyme-linked immunosorbent assay; *g6pd*: Glucose-6-phosphate dehydrogenase; HIV: Human immunodeficiency virus; IGRA: Interferon-gamma release assay; IC: Immunocompetent; IFAT: Immunofluorescence antibody test; IFN-γ: Interferon-gamma; IL-2: Interleukin-2; IP-10: Interferon gamma-induced protein 10; ITS: Internal transcribed spacer; IS: Immunosuppressed; kDNA: Kinetoplast DNA; LAMP: Loop-mediated isothermal amplification; LST: Leishmanin skin test; MeSH: Medical Subject Headings; MIG: Monokine induced by gamma interferon; NTD: Neglected tropical disease; PCR: Polymerase chain reaction; PKDL: Post-kala-azar dermal leishmaniasis; PRISMA-P: Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols; qPCR: Quantitative polymerase chain reaction; RDT: Rapid diagnostic test; rK26: Recombinant kinesin 26; rK28: Recombinant kinesin 28; rK39-RDT: Recombinant kinesin 39 rapid diagnostic test; SOT: Solid organ transplant; sCD40L: SolubleCD40 ligand; *ssu*18S rRNA: Small subunit 18S ribosomal RNA; TNF: Tumor necrosis factor; VL: Visceral leishmaniasis; WB: Western blot; WBA: Whole blood assay; WHO: World Health Organization.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13071-021-05129-y.

Additional file 1: Table S1. List of countries considered endemic.

Additional file 2: Table S2. Search syntax for PubMed.

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Authors' contributions
AVIM and CFP conceptualized the study. AVIM, AC, VW, CO and CFP contributed to the study concept. AVIM and CO designed the protocol. AVIM, AC, VW and CO wrote the manuscript under the supervision of CFP. All authors read and approved the final manuscript.

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Declarations

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Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no financial or non-financial competing interests.

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